Global Journal of Medical Research

discovering thoughts and inventing future

Volume 10 Issue 1 Version 1.0

ISSN: 0975-5853

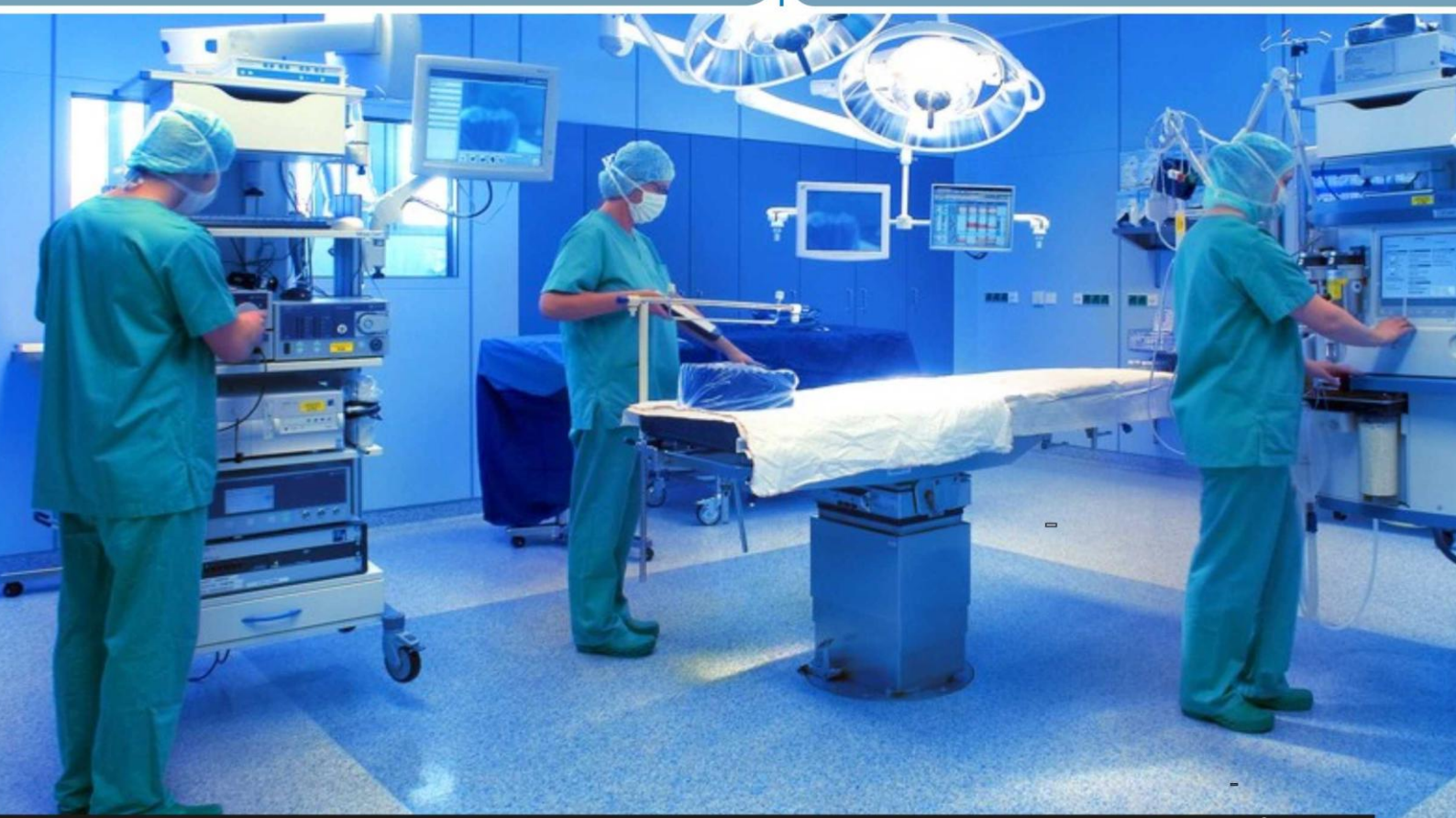
8 Expeditions In Medical World

The Volume 10

Issue 1 Version 1.0

highlights

- Solid Psuedo Papillary Tumour
- Auditory Plasticity
- Kernal Based off-Target Analysis
- Retarding Myopia Progression



Global Journal of Medical Research

Global Journal of Medical Research

Volume 10 Issue 1 (Ver. 1.0)

Global Academy of Research and Development

License No.: 42125/022010/1186
Registration No.: 430374
Import-Export Code: 1109007027

Reviewer, Research and Analysis Centre

Global Journals, Headquarter Office, United States

Offset Typesetting

Global Journals, City Center Office, United States

Financial and Continental Dispatching

Global Journals, Data warehouse, India

Pricing (Including by Air Parcel Charges):

For Authors:
22 USD (B/W) & 50 USD (Color)

Yearly Subscription (Personal & Institutional):
200 USD (B/W) & 500 USD (Color)

Editorial Board Members

Dr. Carlos García Pont
Associate Professor of Marketing
IESE Business School, University of Navarra
Doctor of Philosophy (Management),
Massachussetts Institute of Technology (MIT)
Master in Business Administration, IESE,
University of Navarra
Degree in Industrial Engineering,
Universitat Politècnica de Catalunya

Dr. Fotini Labropulu
Mathematics - Luther College
University of Regina
Ph.D., M.Sc. in Mathematics
B.A. (Honors) in Mathematics
University of Windsor

Dr. Lynn Lim
Reader in Business and Marketing
Roehampton University, London
BCom, PGDip, MBA (Distinction), PhD, FHEA

Dr. Mihaly Mezei
ASSOCIATE PROFESSOR
Department of Structural and Chemical Biology
Mount Sinai School of Medical Center
Ph.D., Etvs Lornd University
Postdoctoral Training, New York University

Dr. Miguel Angel Ariño
Professor of Decision Sciences
IESE Business School
Barcelona, Spain (Universidad de Navarra)
CEIBS (China Europe International Business School).
Beijing, Shanghai and Shenzhen
Ph.D. in Mathematics
University of Barcelona
BA in Mathematics (Licenciatura)
University of Barcelona
Philip G. Moscoso
Technology and Operations Management
IESE Business School, University of Navarra
Ph.D in Industrial Engineering and Management, ETH Zurich
M.Sc. in Chemical Engineering, ETH Zurich

Dr. Sanjay Dixit, M.D.
Director, EP Laboratories, Philadelphia VA Medical Center
Cardiovascular Medicine - Cardiac Arrhythmia
Univ of Penn School of Medicine

Dr. Han-Xiang Deng
MD., Ph.D
Associate Professor and Research Department
Division of Neuromuscular Medicine
Davee Department of Neurology and Clinical Neurosciences
Northwestern University Feinberg School of Medicine

Dr. Pina C. Sanelli
Associate Professor of Public Health
Weill Cornell Medical College
Associate Attending Radiologist
NewYork-Presbyterian Hospital
MRI, MRA, CT, and CTA
Neuroradiology and Diagnostic Radiology
M.D., State University of New York at Buffalo,School of Medicine and Biomedical Sciences

Dr. Roberto Sanchez
Associate Professor
Department of Structural and Chemical Biology
Mount Sinai School of Medicine
Ph.D., The Rockefeller University

Dr. Wen-Yih Sun
Professor of Earth and Atmospheric SciencesPurdue University Director
National Center for Typhoon and Flooding Research, Taiwan
University Chair Professor
Department of Atmospheric Sciences, National Central University, Chung-Li, TaiwanUniversity Chair Professor
Institute of Environmental Engineering, National Chiao Tung University, Hsin-chu, Taiwan.Ph.D., MS The University of Chicago, Geophysical Sciences
BS National Taiwan University, Atmospheric Sciences
Associate Professor of Radiology

Dr. Michael R. Rudnick
M.D., FACP
Associate Professor of Medicine
Chief, Renal Electrolyte and Hypertension Division (PMC)
Penn Medicine, University of Pennsylvania
Presbyterian Medical Center, Philadelphia
Nephrology and Internal Medicine
Certified by the American Board of Internal Medicine

Dr. Bassey Benjamin Esu
B.Sc. Marketing; MBA Marketing; Ph.D Marketing
Lecturer, Department of Marketing, University of Calabar
Tourism Consultant, Cross River State Tourism Development Department
Co-ordinator , Sustainable Tourism Initiative, Calabar, Nigeria

Dr. Aziz M. Barbar, Ph.D.
IEEE Senior Member
Chairperson, Department of Computer Science
AUST - American University of Science & Technology
Alfred Naccash Avenue – Ashrafi

Chief Author

Dr. R.K. Dixit (HON.)
M.Sc.,Ph.D.,FICCT
ChiefAuthor,India
Email: cheifauthor@globaljournals.org

Dean & Editor-in-Chief (HON.)

Vivek Dubey (HON.)
MS(IndustrialEngineering),
MS(MechanicalEngineering)
University of Wisconsin
FICCT
Editor-in-Chief, USA
editorusa@computerresearch.org

Sangita Dixit
M.Sc., FICCT
Dean and Publisher, India
deanind@computerresearch.org

Er. Suyog Dixit
BE (HONS. in Computer Science), FICCT
SAP Certified Consultant
Technical Dean, India
Website: www.suyogdixit.com
Email:suyog@suyogdixit.com,
dean@computerresearch.org

Contents of the Volume

From the Chief Author's Desk

We see a drastic momentum everywhere in all fields now a day. Which in turns, say a lot to everyone to excel with all possible way. The need of the hour is to pick the right key at the right time with all extras. Citing the computer versions, any automobile models, infrastructures, etc. It is not the result of any preplanning but the implementations of planning.

With these, we are constantly seeking to establish more formal links with researchers, scientists, engineers, specialists, technical experts, etc., associations, or other entities, particularly those who are active in the field of research, articles, research paper, etc. by inviting them to become affiliated with the Global Journals.

This Global Journal is like a banyan tree whose branches are many and each branch acts like a strong root itself.

Intentions are very clear to do best in all possible way with all care.

Dr. R. K. Dixit
Chief Author
chiefauthor@globaljournals.org

Solid Pseudo Papillary Tumor of The Pancreas. A Rare Indolent Pancreatic Tumor

Tharwat Kandil (MD)[1] Khaled Zalata (MD)[2]

GJMR Classification (FOR) 321015,321006

***Abstract*- Background/Aim: Solid-pseudo papillary tumor (SPPT) is a very rare primary neoplasm of the pancreas, occurring at all ages; Frantz first described it in 1959. This is a case series study to assess different presentation, diagnostic methods and demographic specifications of this rare disease.**

***Patients and methods*: Six cases of SPPT, admitted from January 2000 to February 2008, were analyzed retrospectively, and most of the literatures concerning SPPT published in the medical journals from January 1994 to March 2008 were analyzed.**

***Results*: From January 2000 to February 2008, Six patients were operated in our center with final pathologic diagnosis of SPPT. The group consisted of five females and one male (Mean age =24.8 years). The tumors were ranging in diameter from 6-12 Cm. These were partially cystic. All the tumors exhibited variable degree of papillary pattern. Cytokeratin was nearly diffuse positive. Alpha-1-antitrypsin and vimentin were diffusely positive, cytoplasmic reaction, while synaptophysin is focally positive. There were no mortality and our patients still survived in good health with no evidence of any tumor recurrence. The last patient done 24 months before writing this paper while the first case survived 8 years.**

Conclusion: SPPT of the pancreas is a rare indolent neoplasm with an unclear origin that typically occurs in young females. The diagnosis depends on histological confirmation. Surgical resection has generally been curative, but close follow up is recommended, particularly when the histological appearance suggests a more aggressive tumor

I. Introduction

Solid-pseudo papillary tumor (SPPT) is a very rare primary neoplasm of the pancreas (2-3% of primary pancreatic tumors) occurring at all ages, Frantz first described it in 1959[1]. It is a disease with a low-grade malignant potential affecting predominantly adolescent girls and young women. In recent years, the incidence has been increasing [2].

The Tumor has given several different names according to its macroscopic and microscopic pathological characteristics. Examples of this names, papillary-cystic tumor, solid cystic tumor, papillary epithelial neoplasm Solid and papillary neoplasm, papillary tumor of the pancreas, or Frantz's tumor) until a consensus was reached in 1996, when it was defined by the World Health Organization (WHO) as a unique tumor with its current name: solid pseudo papillary tumor of the pancreas [3] Due to the cell characteristics of SPPT, different origin hypotheses have been postulated. Many investigators favor the theory that SPPT originate from multi potent primordial cells while others suggest an extra-pancreatic origin from genital ridge angle-related cells. Some controversy exists for both hypotheses. It could consider their origin to be from pluripotent embryonic cells of the pancreas with multi potential differentiation. In fact, on the one hand, STPs express epithelial, mesenchymal, exocrine and endocrine features, and their cells may originate from the ductular-centroacinar cell compartment which, during embryogenesis, is thought to give rise to exocrine and endocrine cells. However, on the other hand, there is no evidence of clear-cut terminal differentiation to either acinar or endocrine cells; the cytological features and the low proliferative activity and malignancy are not consistent with a stem cell origin and, finally, the strongly sex-linked occurrence is not in keeping with a stem cell origin [4].

It is a tumor that, despite possible histological findings of malignancy, has a benign clinical behavior with low malignant Potential [5].

SPPTs characterized by unique clinical and pathological features. They are most common in females (90%) and occur predominantly at a young age (mean age 35 years with less than 10% over 40 years) .They are more frequent in non-Caucasians with the highest incidence in Japan . They regarded as slow-growing relatively benign tumors, though metastasis has been reported [6].Even in the presence of disseminated disease, the clinical course is usually favorable [7].

A. Materials and Methods

This is a descriptive and retrospective study of patients diagnosed by computed tomography scanning and then treated with surgery in the Gastro-intestinal surgical center, Mansoura University, Egypt, January 2000 to February 2008. Most of the literatures concerning SPPT published in the medical journals from January 1994 to March 2008 were analyzed.

We reviewed the patients' files with pathology report of pseudo papillary tumor of pancreas that were operated in our center from January 2000 to February 2008. We collect and record these data: age, sex, signs and symptoms, past medical history, physical examinations, pre-operative

About[1]- Gastroenterology Surgical Center, Faculty of Medicine, Mansoura University, Jihan St., Mansoura, Egypt
(e-mail tharwat@mans.edu.com)
About[1] - professor, Gastroenterology Surgical Center, Mansoura University, Egypt (e-mail: – info@th-kandiel.com(Tel 0020 10 578 1960)

diagnostic measures, laboratory data, per-operative findings, macroscopic and microscopic features of tumors, H&E, Masson trichrom as well immunohistochemical staining with synaptophysin, cytokeratin (AE1/AE3), vimentin and alpha-1-antitrypsin was performed Monoclonal antibodies for synaptophysin, cytokeratin (AE1/AE3) and vimentin as well polyclonal alpha-1-antitrypsin antibody were applied from Dako Corp. Antibodies were applied for 1 hour at room temperature; Aviden-Biotin system and DAB as chromogen were applied. Post-operative follows up of the patients. In the follow up period, the patients were visited in planned intervals (after 1 month, 6 months, and then every 6 months) by the operating surgeon. A control computed tomography (CT) scan with oral and intravenous contrast was performed 6 months after the surgery. Finally, as the tumor is rare, we try to discuss our results with regards to other reported series.

B. Results

From January 2000 to February 2008, Six patients were operated in our center with final pathologic diagnosis of (SPPT), (out of 360 cases of pancreatic and periampulary tumor operated upon in the same period). The group consisted of five females and one male at the age of 30, 14, 30, 25, 23 and 27(Mean =24.8 years). The disease was presented as right upper quadrant abdominal pain in three cases and in epigastrium in three cases and as an abdominal enlargement in one case , loss of weight in 2 cases and as jaundice in one (with exploration and T- Tube 2 wks before admission in our center).

Physical examination revealed no positive abdominal finding apart from mild epigastric fullness in five cases while one presented 2 wks post operative after exploration for jaundice. An abdominal ultrasound showed a cystic solid mass which was followed by a spiral abdominal and pelvic CT scan with oral and intravenous contrast that confirmed a solid mass with gas inside and cystic areas with areas of calcification in right side of upper abdomen in 3 cases, Picture 1, in the left side in one case and in the pancreatic head region in 2 cases.

We did not try to take a tissue diagnosis in our cases; this is the concept in our center. Laboratory data including complete blood count, blood chemistry, serum amylase level and coagulation profiles were normal in all cases apart from mild hyperbilirubinemia in one case.

Pathological findings: The tumors were ranging in diameter from 6-12 Cm, Picture 2. These were partially cystic. All the tumors exhibited variable degree of papillary pattern (Fig.1). Cytokeratin was nearly diffuse positive (Fig. 2). Alpha-1-antitrypsin (Fig. 3) and vimentin were diffusely positive, cytoplasmic reaction, while synaptophysin (Fig. 4) is focally positive.

The extend of resection in five patients was whipple's operation, distal pancreatectomy and splenectomy in the one case.

Postoperatively patients were hospitalized for 6-8days and then discharged without complications. Follow up period times were 24, 30, 60, 66, 48 and 36 months. There was no abnormality in control CT scans.

There were no mortality and our patients still survived in good health with no evidence of any tumor recurrence by follow up CT. the last patient done 24 months before writing this paper while the first case survived since 8 years.

Table1: Case series patient demonstrations

Case Number	sex	age	Patient complaint	Tumor size	Extent of surgery
1	Female	30	RUQP & Epig P	5*5*7	Whipple, Op
2	Female	14	RUQP & Wt Loss	7*7*5	Whipple, Op
3	Female	30	RUQP	5*4*6	Whipple, Op
4	male	25	Epig pain & abd Enlargement	8*9*5	distal pancreatectomy & splenectomy
5	Female	23	jaundice	5*5*7	Whipple, Op
6	Female	27	Epig pain & Wt Loss	6*6*8	Whipple, Op

II. Discussion

SPPT of the pancreas is a rare exocrine pancreatic tumor, which comprises only 1%-2% of all tumors of the pancreas, first described by Frantz in 1959. Recently, Papavramidis et al reviewed 210 English language papers published from 1933 to 2003, and found 718 well-documented cases [8].Solid-pseudo papillary tumor is almost exclusively (90%) encountered in young females between the 2nd and 4th decades. The body and tail of the pancreas are more frequently affected (64%) than the head. These tumors are detected incidentally in most cases; however, abdominal pain may be the sole and important sign of the tumor. Additionally, abdominal mass, anorexia, weight loss, and symptoms due to the compression of adjacent organs can be present [9].This Findings is in concordance with our series as we find the disease affecting 80 % female and 20% in male and most of the cases presented with vague right upper quadrant abdominal or epigastric pain while an abdominal enlargement in one case , loss of weight in 2 cases and as jaundice in one.

The imaging technique most widely used in its diagnosis is CT scanning, and there is a characteristic –though nonspecific– pattern. In radiographic studies, the presence of a large hyper vascular encapsulated mass is noted in contact with the pancreatic tissue, with peripheral contrast enhancement. It may show either a solid or cystic aspect, or be heterogeneous in appearance. The existence of various layers of cystic tissue will correspond to the solid tissue necrosis found in the pathologic study. Occasionally, calcifications and bleeding signs may be seen inside the tumor. Differentiating SPTPs from adenocarcinomas is crucial, as the latter's prognosis is much worse. MRI images show characteristics similar to those of CT, enabling a clearer picture of bleeding areas. Currently there is an increased use of echo-endoscopy with fine-needle puncturing in the diagnosis of this pancreatic neoplasm, which are especially useful for cystic lesions . On certain occasions this may help in the study of the tumor [10].In our center, we depend mainly on thin cuts abdominal CT scan with IV and oral contrast to diagnose pancreatic lesions and on post operative pathology.

Although, some radiological signs are suggestive of SPPT, radiological guide FNAC may be needed to obtain a preoperative diagnosis. We depend on radiological findings in the diagnosis of pancreatic cancer, but we confirm the diagnosis by postoperative pathology. In one study reviewing over 150 cases of SPPT, when preoperative FNAC was done, over 70% of lesions were definitely diagnosed as SPPT or had SPPT or low-grade epithelial neoplasm in the differential diagnosis [11].

Grossly, they present as large, round, solitary masses (range: 3 - 18 cm) that are usually well demarcated from the remaining pancreas. The cut surface of the tumors reveals lobulated, light brown solid areas admixed with zones of hemorrhage and necrosis as well as cystic spaces filled with necrotic debris. However, cystic changes can be less prominent in smaller tumors [12].In our series, the tumors ranging in diameter from 6-12 Cm, these were partially cystic. They are usually solitary tumors which are evenly distributed throughout the pancreas though unusual multi centric .or extra pancreatic (mesocolonic, retroperitoneal, omental, hepatic) presentations have been reported.

The microscopic features of SPT are solid areas, which alternate with a pseudopapillary pattern composed of a fibrovascular stalk surrounded by several layers of epithelial cells. Immunohisto chemical studies are frequently performed to confirm the diagnosis. SPT is typically positive for vimentin, and antitrypsin and are negative for trypsin and chymotrypsin. They may also show focal immunoreactivity for neuron-specific enolase (NSE) and cytokeratin [13, 14].

In Our series, Post operative Microscopic Examination of the specimens revealed that Alpha-1-antitrypsin (Fig 3) and vimentin are diffusely positive, cytoplasmic reaction. This is in concordance with Pettinato et al, 1992[15].

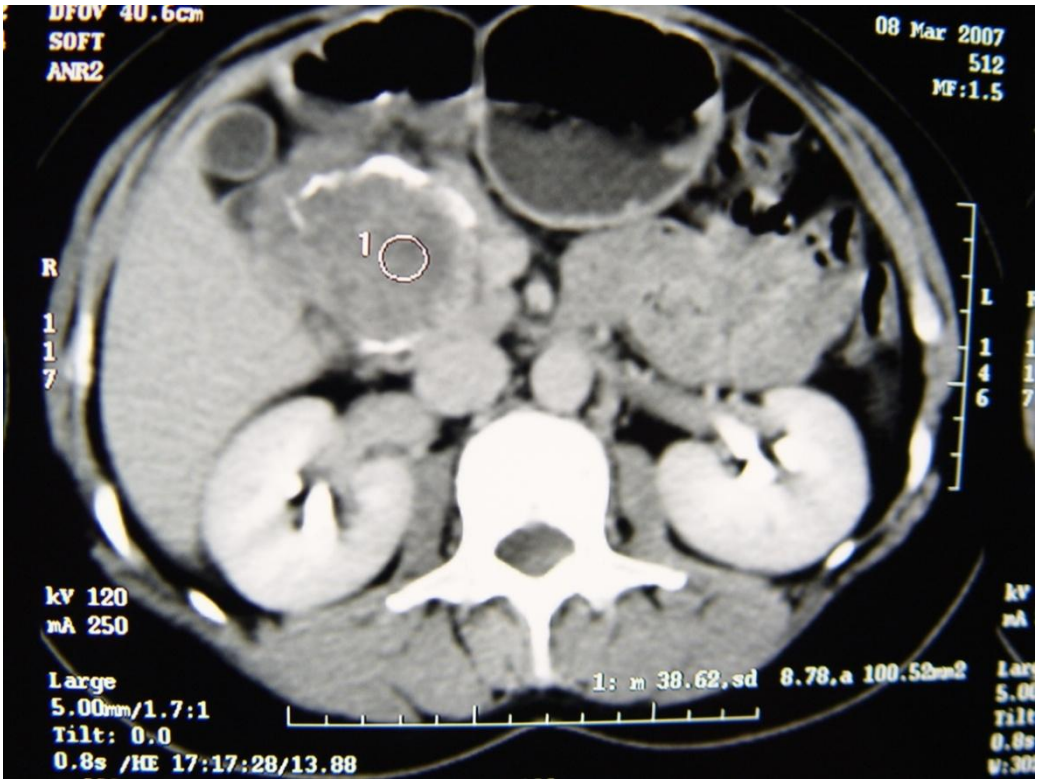
Cytokeratin is nearly diffuse positive, that is similar to findings of Pettinato et al, 2002 while synaptophysin is focally positive [16].

Surgery is the mainstay of treatment, which is usually curative for localized disease. There is evidence for prolonged survival after adequate surgical resection even with metastases. Even if the disease is extensive at the time of presentation, surgical debulking favors prolonged survival Intra-operative frozen section may be helpful to ascertain the adequate of the resection margins. There have been only few reports of the use of radiotherapy or chemotherapy so it is difficult to judge the value of such measures [17].

Although the majority of these tumors considered being of low malignant potential, a recent report of two cases of aggressive SPPTs noted death of the patients at 6 and 16 months after diagnosis. In these cases a high mitotic index and extensive tumor necrosis was noted. These findings might help distinguish a more virulent form of SPPT and lead to a more aggressive post-operative treatment course [18].In our series, no such cases encountered.

In conclusion, SPPT of the pancreas is a rare indolent neoplasm with an unclear origin that typically occurs in young females. The diagnosis depends on histological confirmation, but its appearance on imaging is fairly characteristic. Surgical resection has generally been curative, but close follow up is recommended, particularly when the histological appearance suggests a more aggressive tumor.

Picture 1 CT finding in patient with SPPT



Picture 2 macroscopic picture of SPPT

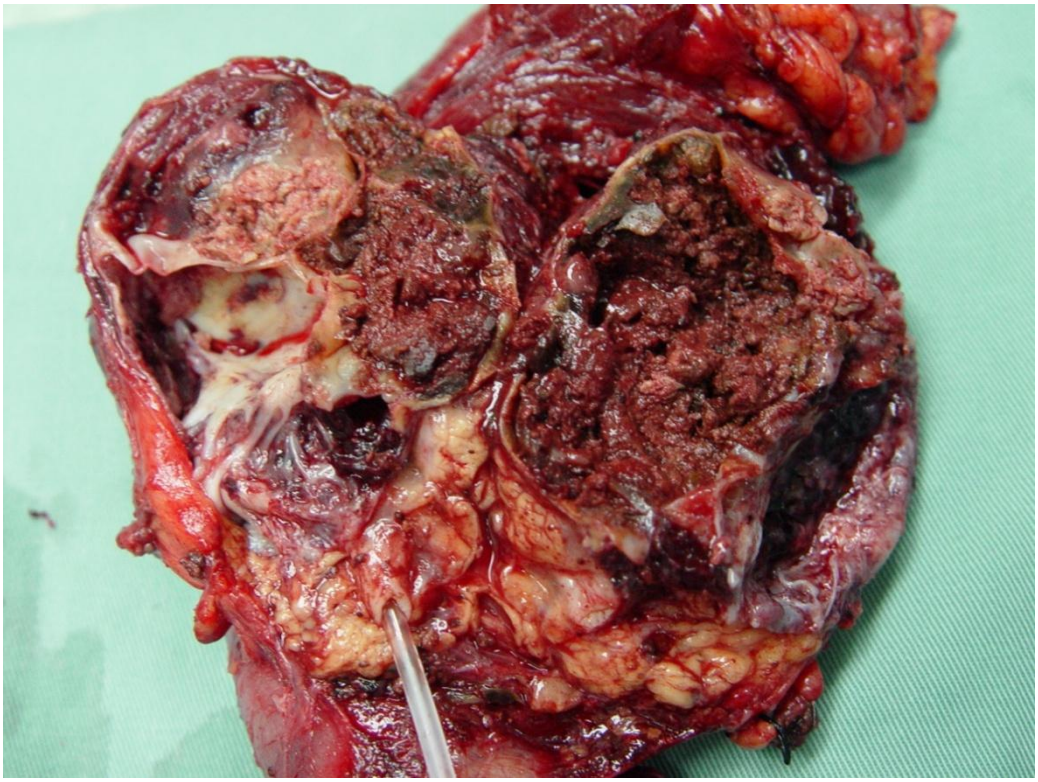


Fig. 1

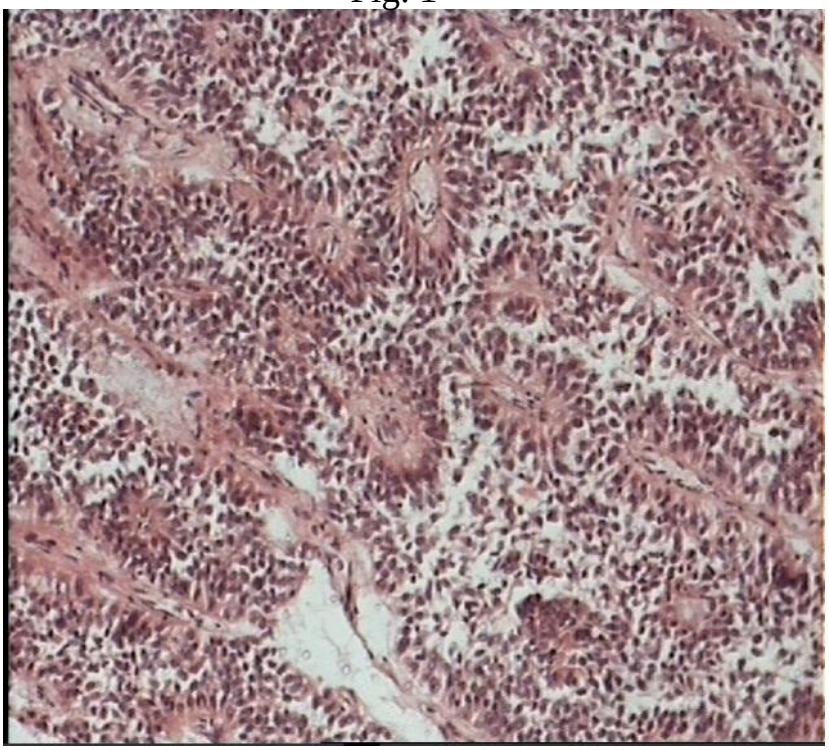


Fig. 2

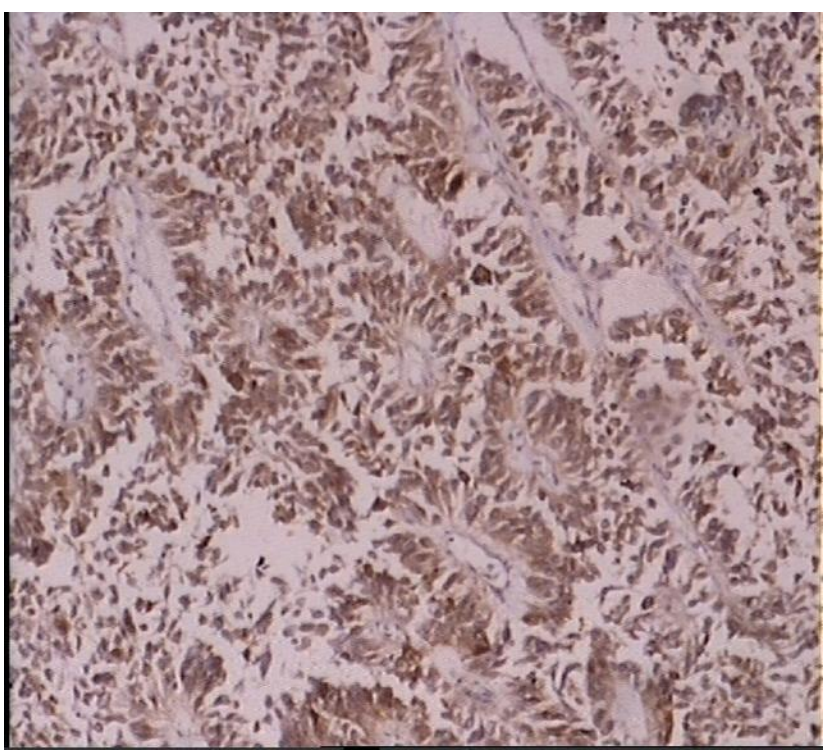


Fig. 3

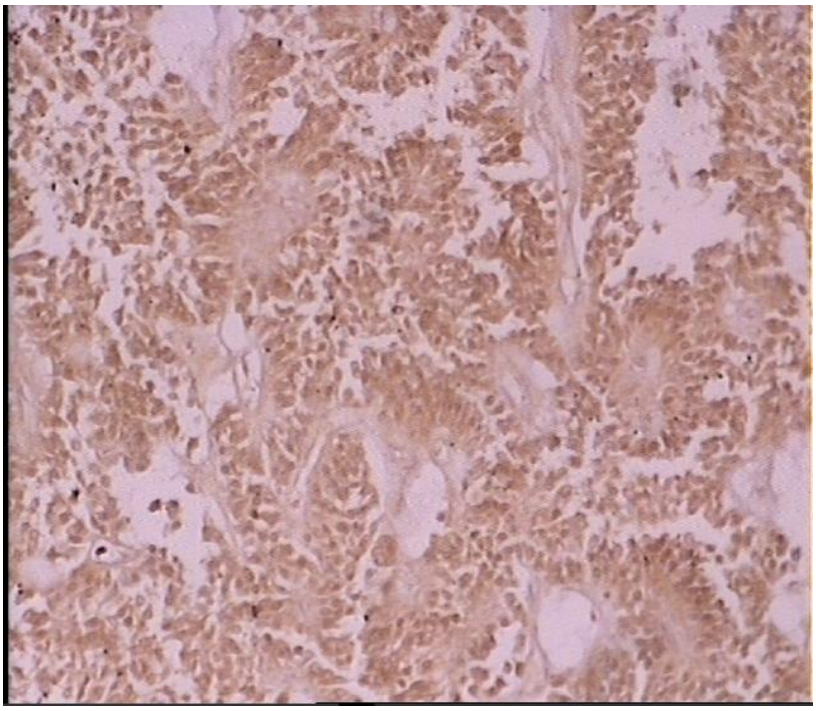
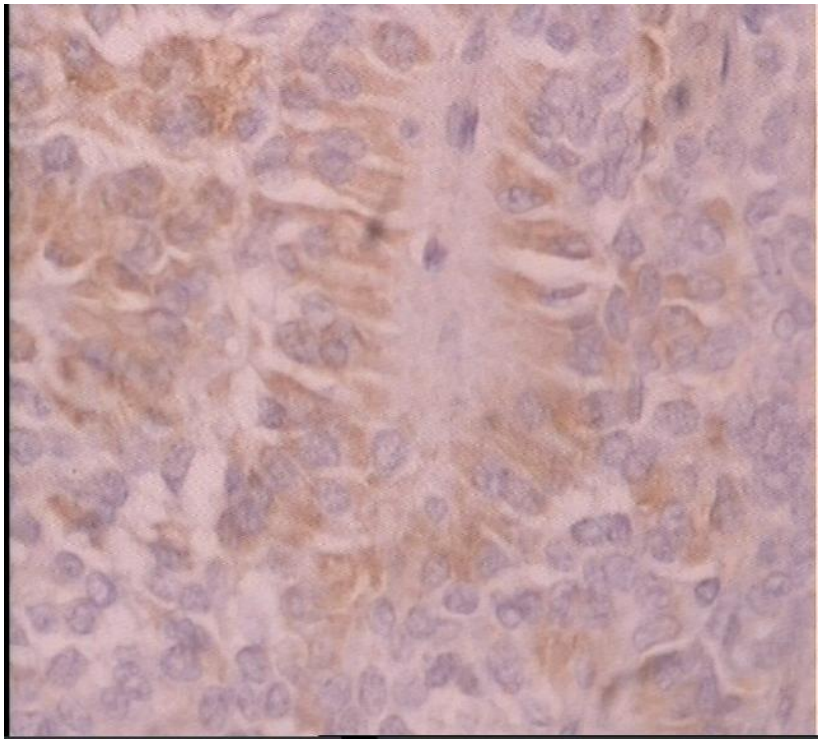


Fig. 4



III. References

1) Morohoshi T, Held G, Kloppel G. Exocrine pancreatic tumors and their histological classification: a study based on 167 autopsy and 97 surgical cases. Histopathology 1983; 7: 645-61.
2) Chun-Yi Hao, Ai-Ping Lu, Bao-Cai Xing, Xin-Fu Huang, Fei Gao, Jia-Fu Ji. Solid Pseudopapillary Tumor of the Pancreas: Report of 8 Cases in a Single Institution and Review of the Chinese Literature. pancreatology 2006;6:291-296.
3) Kloppel G, Solcia E. Longnecker DS, Capella C, Sobin LH. World Health Organization institutional histological classification of tumors– histological typing of tumors of the exocrine pancreas. 2nd ed. Berlin, Germany: Springer-Verlag, 1996.
4) Donatella Santini, Francesca Poli, Stefania Leg. Solid-Papillary Tumors of the Pancreas: Histopathology.JOP. J Pancreas (Online) 2006; 7(1 Suppl.):131-136.
5) Yamanue H, Tanimura H, Shono Y, Onishi H, Tani M, Yamoto H, et al. Solid and cystic tumor of the pancreas: clinicopathologic and genetic studies of four cases. Int J Pancreatol 2000; 27 (1): 69-76.
6) Lam KY, Lo CY, Fan ST. Pancreatic solid-cystic-papillary tumor: clinicopathologic features in eight patients from Hong Kong and review of the literature. World J Surg 1999; 23:1045-50
7) Martin RC, Klimstra DS, Brennan MF, Conlon KC. Solid-pseudopapillary tumor of the pancreas: a surgical enigma? Ann Surg Oncol 2002; 9: 35-40
8) Hao Zhang, Ting-Bo Liang, Wei-Lin Wang, Yan Shen, Guo-Ping Ren and Shu-Sen Zheng. Diagnosis and treatment of solid-pseudopapillary tumor of the pancreas: Hepatobiliary Pancreat Dis Int2006; Vol 5, No 3 • August 15.
9) Erdal KARAGÜLLE, Erkan YILDIRIM, Emin TÜRK, Halil KIYICI, Hamdi KARAKAYALI. Solid pseudopapillary tumor of the pancreas: A case report. Turk J Gastroenterol 2006; 17 (4): 316-319.
10) Frago R, Fabregat J, Jorba R, García-Borobia F, Altet J, Serrano MT, Valls C. Solid pseudopapillary tumors of the pancreas: diagnosis and curative treatment. Rev Esp Enferm Dig 2006; 98: 809-816.
11) Crawford BE 2nd. Solid and papillary epithelial neoplasm of the pancreas, diagnosis by cytology. South Med J 1998; 91: 973-977
12) Jai Hyang Go: A Spindle Cell Predominant Pancreatic Solid- pseudopapillary Tumor. Yonsei Med J 2008;49(4):672 - 675,
13) Santini D, Poli F, Lega S. Solid-papillary tumors of the pancreas: histopathology. JOP 2006; 7:131-136.
14) Egawa S, Sunamura M, Abe H, Motoi F, Fukuyama S, Matsuno S. Clinicopathological aspects of pancreatic cancer. Gan To Kagaku Ryoho 2005;32:605-611.
15) Pettinato G, Manivel JC, Ravetto C et al. Papillary cystic tumour of the pancreas: A clinicopathologic study of 20 cases with cytologic, immunohistochemical, ultrastructural and flow cytometric observations and a review of the literature. Am J Clin pathol 1992; 98:478-488.
16) Pettinato G, Di Vizio, Manivel JC, et al. Solid-pseudopapillary tumour of the pancreas: a neoplasm of distinct and highly characteristic cytologic features. Diagn Cytopathol 2002; 27: 325-334.
17) Fried P, Cooper J, Balthazar E, Fazzini E, Newall J. A role for radiotherapy in the treatment of solid and papillary neoplasms of the pancreas. Cancer 1985; 56: 2783-2785.
18) Tang LH, Aydin H, Brennan MF, Klimstra DS. Clinically aggressive solid pseudopapillary tumors of the pancreas: a report of two cases with components of undifferentiated carcinoma and a comparative clinicopathologic analysis of 34 conventional cases. Am J Surg Pathol 2005; 29: 512- 519.

Laparoscopy with Augmented Reality Technology

Mauricio Plaza Torres

Mplaza200@yahoo.com

GJMR Classification (FOR)
321029,321099

Abstract- **Laparoscopy technique has caused a high impact in the surgical community. The success of this technology is the application of the surgical conventional practices to access in the abdominal cavity, with diagnostic and therapeutic purposes, with a minimum surgical intervention. This procedure has been used for biliary lithiasis, now it is an advanced procedure in several pathologies interventions; the reason is because surgical set of instruments has been improving. The base of laparoscopy procedure is the video surgery, which for nature needs the training of the surgeon and the knowledge of the set of instruments, as well as his advantages and his limitations.**

The correct use of this technology prevents surgical problems and the correct knowledge of the instruments in every particular condition, avoids human operation mistakes. Additionally to all inherent problems in the use of the technology, an additional variable exists; the position of the image is displayed in a monitor, in a different position from the area of operation. This implies an additional training of the surgeon so that the surgical act has fluency and continuity.

The image is taken by an endocamera and projected in a monitor. The first barrier that the surgeon must overcome is the difference between the position of the image and the the same characteristics of an open surgery, we can have closer place of navigation of the instruments. If there is the possibility of projecting the image on the human body with this methodology over to the traditional surgeries, achieving the advantages of the video surgery over a virtual traditional surgery. With this new technological modification, the surgeon does not use the image of monitor and in his place he sees, in his visual field, the internal image of the abdomen like an open traditional surgery.

The training would centre on the use of the laparoscopy instruments and the surgeon will feel the sensation of being in a traditional operation, with consistent progress in his surgical skill. In order to obtain this objective, we can propose the use of the augmented reality technique, which allows having the human real body in the field of vision and the superposition of the internal image of the abdomen [4].

I. General Objective

The objective is to improve the laparoscopy procedure skills by means of the use of augmented reality devices, projecting in virtual way, the image of the endocámara in the position of laparoscopy devices navigation, making the procedure more natural, bringing this technology to the traditional surgery with his consistent benefits (Fig 1).

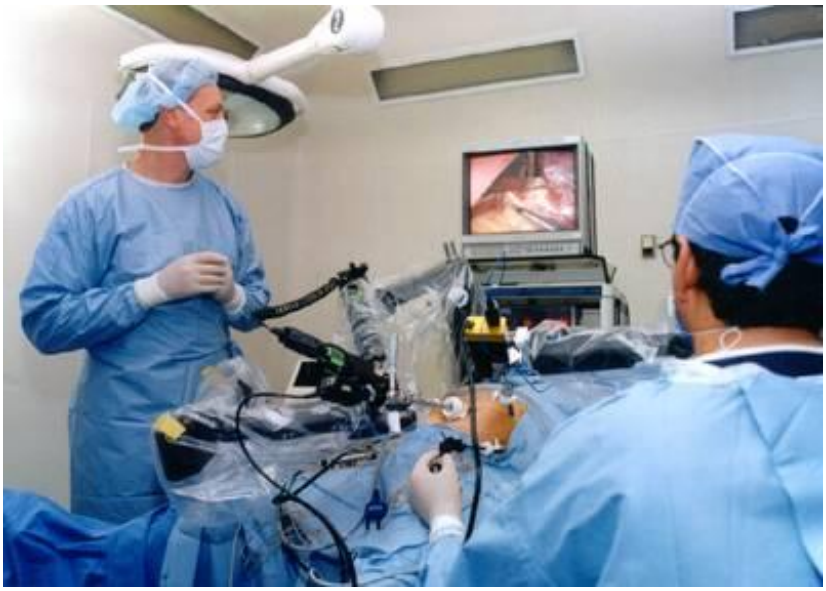


Fig 1 Tradicional laparoscopy technology

II. Specific objetives

• Connect the traditional surgery laparoscopy system to the augmented reality system
• Create a prototype adapted to the surgery laparoscopy training systems.
• Determine the grade of ergonomics and satisfaction for the use of this prototype for surgeons and doctors in training.
• Realize the final prototype for surgery in Pigs.
• Determine the grade of ergonomics and satisfaction for the use of this prototype in training for doctors and surgeons in practices with Pigs.
• Realize the final prototype.

III. Justification

The laparoscopy surgery procedure represents an important advance in the procedures by the surgical community. The technique has already begun several decades ago by gynecologists and now it is applied to the general surgical practices, for the reason of the advances in the surgical instrumentation and the medical equipment electronics.
With the purpose of improving the malpractice statistics and surgical errors, the project suggest adding new techniques to the procedure in order to improve the devices using modern techniques like augmented reality [1] (Fig 2).

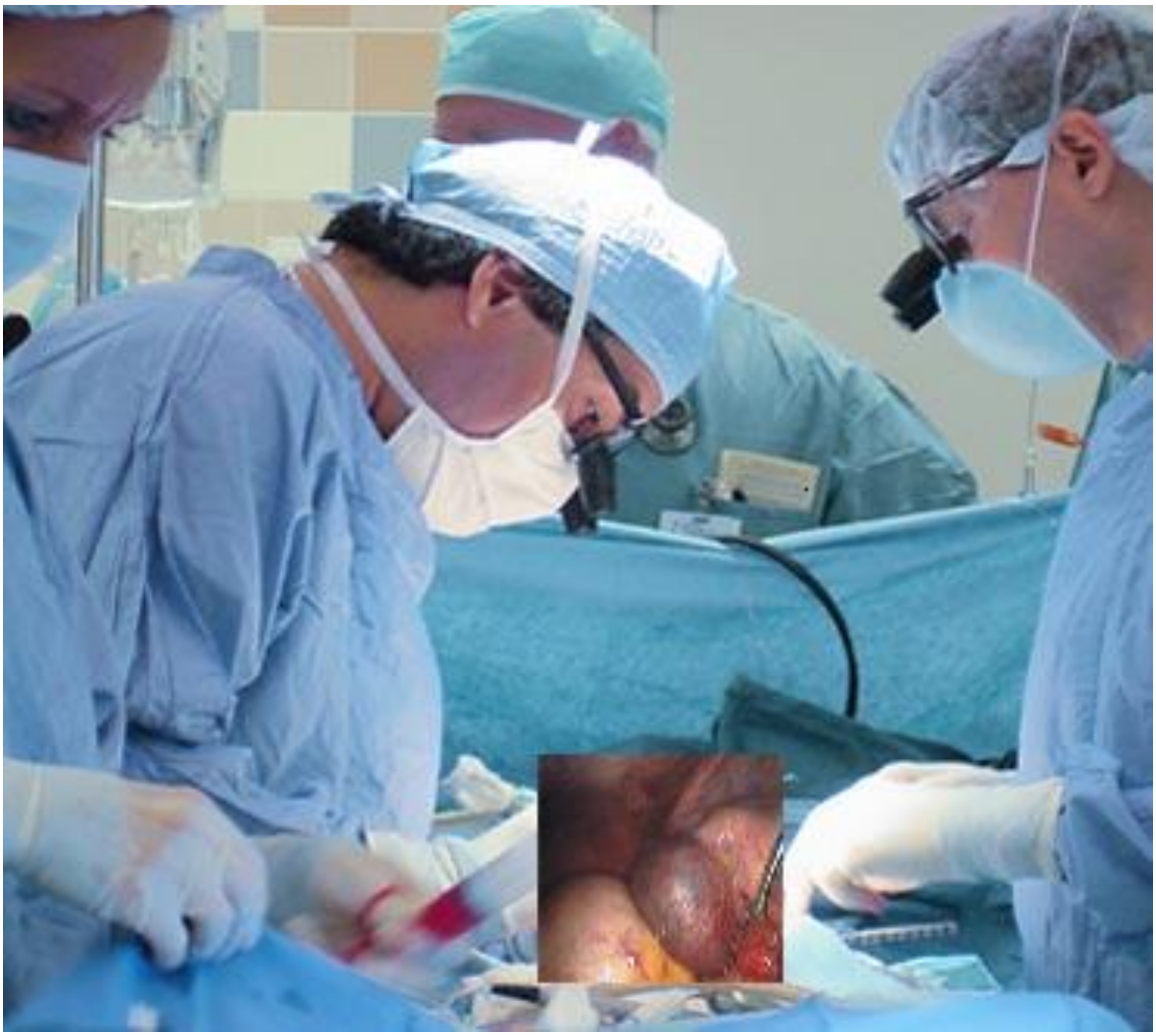


Fig 2. Laparoscopy surgery with the suggested augmented reality technology

The basic idea consists of using the augmented reality to project the endocámara image on the patient abdomen, simulating to the surgeon a traditional open surgery operation. Projecting the image on the human body with the same characteristics of an open surgery, we can have closer this methodology over to the traditional surgeries, achieving the advantages of the video surgery in a virtual traditional surgery. This proposed system generates

comfort and fluency in the surgical procedure, improving the interface man-machine with benefits in the procedure [2].

IV. Methodological Approach

1) Bibliographical review. It allows knowing the advances in systems of augmented reality and his possible applications in laparoscopy surgery.
2) Connect the system of traditional laparoscopy to the equipment of augmented reality.
3) Select the type of reflective or electronic technology (Optical See-Through or Video See-Through) [3]
4) Creation of a prototype of augmented reality applied to laparoscopy training equipment.
5) Realize tests to determine the grade of satisfaction and of progress in the procedures of training of the medical personnel.
6) Improve the device in accordance with the results of the tests
7) Use the improved prototype for tests in Pigs
8) Realize tests to determine the grade of satisfaction and of progress in the training procedures of medical personnel.
9) improve the device in accordance with the results of the tests
10) Produce the final prototype.

V. EXPECTED RESULTS

The final prototypes improve the surgery laparoscopy procedure, allowing the surgeon to realize interventions with the same visual characteristics of a traditional open surgery operation. This proposed system generates comfort and fluency in surgical laparoscopy procedures, improving the interface man-machine with benefits in procedure, helping to reduce, in combination with other parameters, latrogenias and intraoperative fails.

VI. Reference

1) Bowman, D.A.[Doug A.], McMahan, R.P.[Ryan P.],Virtual Reality: How Much Immersion Is Enough?,IEEE Computer No. 7, July 2007, pp. 36-43
2) Riva, G. (2005), "Virtual reality in psychotherapy: review", Cyberpsychology & Behavior, v. 8, n. 3, p. 220-230.
3) Siegl, H., Hanheide, M.[Marc], Wrede, S.[Sebastian], Pinz, A., (2007) An augmented reality human-computer interface for object localization in a cognitive vision system, Image and Vision Computing , No. 12, 3 December 2007, pp. 1895-1903.
4) Winn, W.D. (2005). What We Have Learned About VR and Learning and What We Still Need to Study. Laval Virtual 2005.

Auditory Plasticity. Does It Really Exist? A Preliminary Study

Sachin Sakhuja[1](Maslp)Sanjay Munjal[2]
(PhD)Naresh K. Panda[3](MS)

GJMR Classification (FOR) 321018,320705

***Abstract*- Auditory Plasticity. Does It Really Exist? A Preliminary Study.**

Keywords: Plasticity, Brainstem Evoked Responses, Middle latency Responses, Myelination, Hearing restoration

I. Introduction

Plasticity may be defined as the alternation of nerve cells to better conform to immediate environmental influence. Auditory plasticity refers to the plasticity in the auditory system.

Span of hearing loss approximates the period of auditory deprivation and auditory system doesn't mature without stimulation. Since the re-introduction of stimulation by the hearing aid resumes the normal processing sequence hence if there is something like auditory plasticity there should be some alteration in the capacity for auditory processing which can be revealed by electro physiologic tests.

II. Aims

1- To examine whether any significant changes occur following restoration of hearing by a hearing aid in patients with hearing deprivation.

2- To examine the potential for plasticity in the human auditory system.

III. Methodology

17 patients (10 males and 7 females) in the age range of 10 to 40 years with mild to moderately severe sensorineural hearing loss (unilateral or bilateral) were enrolled for the study. Brain Stem Evoked responses (BSER) and Middle Latency Responses (MLR) studies were conducted on first visit of the patient and hearing thresholds were estimated with the help of Pure tone audiometric (PTA). The patients were made to review for repeated BSER and MLR studies after 2 months of hearing restoration.

IV. Results

In all 17 patients studied, once the hearing was restored with the help of a hearing aid, repeated BSER and MLR studies showed significant decrement in the latencies and improvement in the amplitudes of the waves.

About- *Post Graduate Institute of Medical Education and Research Room no.441, 4th Floor, A block, New OPD Block, Chandigarh, India (160012)*

V. Discussion

Our study reports considerable decrease in the latencies of all the waves ($p>0.01$). Neural conduction time measured through interwave latency also decreased significantly. The possible underlying mechanisms explaining this improvement include improvements in synaptic efficacy and possibly increased myelination.

VI. Conclusion

It can thus be deciphered that, auditory system, retaining its plasticity, if re-stimulated has the capacity to reorganise itself.

VII. Need For The Study

Plasticity may be defined as the alternation of nerve cells to better conform to immediate environmental influence; this alternation is often associated with behavioural change. Auditory plasticity refers to the plasticity in the auditory system1. Development of the human auditory brainstem is thought to be primarily completed by the age of approx 2 years, such that subsequent sensory plasticity is confined primarily to the cortex1.

Whenever the brain has to accommodate new environmental influence, plastic change occurs. The brain is much more plastic in early stages of development through adolescence2. However, even in adulthood, plasticity is evident. Three types of plasticity are seen in the auditory domain. These are developmental plasticity, compensatory plasticity resulting from a lesion somewhere in the CANS and learning related plasticity3. The latter two provide clear evidence of auditory plasticity in the adult auditory cortex. Even when the auditory system is deprived of adequate stimuli for a longer duration of time, human representational cortex has the ability to alter its organization and regain the capacity for auditory processing.

It is still a controversy whether the human system demonstrates similar changes and whether experience with sounds composed of acoustic elements relevant to speech may alter brainstem response characteristics.

Recent studies on learning-related changes in human auditory cortex indicate that auditory cortex seems to process and represent stimuli in a task-dependent fashion which implies plasticity in neural processing4. Further

researchers have revealed experience-dependent developmental plasticity in the mammalian auditory brainstem in an animal model5.

Span of hearing loss approximates the period of auditory deprivation and auditory system doesn't mature without stimulation. Nonetheless, the auditory system retains its A better understanding of the plastic changes in the central auditory system after sensory differentiation, sensory stimulation, and learning may contribute significantly to improvement in the rehabilitation of damaged or lost auditory function and consequently to improved speech processing and production.

VIII. Objectives

The main objectives of the study were:-

1- To examine whether any significant changes occur following restoration of hearing by a hearing aid in patients with hearing deprivation.

2- To examine the potential for plasticity in the human auditory system.

IX. Methodology

17 patients (10 males and 7 females) in the age range of 10 to 40 years having mild to moderately severe sensor neural hearing loss (unilateral or bilateral) , for greater than 2 years, were enrolled for the study conducted at Speech and Hearing Unit, Department of Otolaryngology and Head and Neck Surgery , Post Graduate Institute of Medical Education and Research, Chandigarh, India. The patients with conductive element and/or mixed component contributing to hearing loss which was revealed through pure tone and tympanometry studies were excluded from the study. Patients with any other associated problems were also excluded from the study. The study was passed by the ethical committee of the same institute.

Brain Stem Evoked responses (BSER) and Middle Latency Responses (MLR) studies were conducted on first visit of the patient and hearing thresholds were estimated with the help of Pure tone audio metry (PTA). Moreover, the patient underwent amplification trial and a potential hearing aid as per the loss of the patient was prescribed. The patient was made to review for repeated BSER and MLR studies after 2 months of hearing restoration. Latencies of I,III and V waves and Interpeak latencies I-III, III-V, I-V were estimated at 90 dB nHL before and after use of hearing aid and were further compared. Moreover, amplitudes of Na, Pa and Nb waves were also estimated by conducting middle latency responses (MLR) studies before and 2 months after hearing aid use.

X. Results

In all the 17 patients, once the hearing was restored with the help of a hearing aid, there was significant improvement in the latencies of all the waves established. Morevover, the interpeak latencies also showed decrement after the restoration of hearing.

Table I shows the mean, standard deviation and t values for various parameters assessed through Brain Stem Evoked responses, before and after the hearing restoration

Table I

	Before Mean (SD)	After Mean (SD)	T value
I wave	1.82 (0.25)	1.8 (0.25)	2.40*
III wave	3.69 (0.21)	3.67 (0.20)	3.13**
V wave	5.72 (0.19)	5.69 (0.21)	3.51**
I-III wave	1.86 (0.20)	1.83 (0.19)	3.52**
III-V wave	2.01 (0.19)	1.99 (0.18)	3.00**
I-V wave	3.90 (0.20)	3.88 (0.19)	3.14**

The t values show significant improvement in the latencies of I,III and V waves and I-III, III-V, I-V interpeak latencies after the hearing was restored with the help of a hearing aid.

Table II shows the mean, standard deviation and t values for various parameters assessed through Middle Latency Responses , before and after the restoration of hearing with the help of hearing aid.

Table II

	Before Mean(SD)	After Mean (SD)	T value
Na wave	1.31 (0.67)	1.35 (0.66)	3.12**
Pa wave	1.29 (0.58)	1.34 (0.55)	3.11**
Nb wave	1.28 (0.56)	1.35 (0.54)	3.24**

The t values show significant increase in the amplitudes of Na, Pa and Nb waves after the hearing was restored with the help of a hearing aid

Table III shows the number of patients having different types of tympanograms.

Table III

Impedance Type	Number of patients
Type A	14
Type As	2
Type Ad	1

Table IV shows the status of presence and absence of acoustic reflex.

Table IV

	Number of patients
Reflex present	3
Reflex absent	14

XI. Discussion

Studies report that alterations in the physiological and/or the anatomical properties of the central auditory system (neural plasticity) can be induced by unilateral or bilateral sensor neural hearing loss[7]. The loss of auditory receptors, the hair cells, results in profound changes in the structure and function of the central auditory system, typically demonstrated by a reorganization of the projection maps in the auditory cortex. These plastic changes occur not only as a consequence of mechanical lesions of the cochlea or biochemical lesions of the hair cells by ototoxic drugs, but also as a consequence of the loss of hair cells in connection with aging or noise exposure.[8]

Until recently, researchers used behavioral measures of identification and discrimination of speech and no speech stimuli to assess the effects of auditory deprivation, enhancement, and training. Recent advances in our ability to measure electrical activity in the auditory system in response to sound have made it possible for us to study how changes in auditory input (because of hearing loss, auditory input modification, or training) affect the function of the central auditory system.

Our study reports considerable decrease in the latencies of all the waves ($p > 0.01$) after the restoration of hearing with the help of a hearing aid. Neural conduction time measured through interwove latency also decreased significantly pointing to the capacity of the central auditory system to reorganise itself. Moreover, amplitudes of all the waves observed in Middle Latency responses also showed increment after hearing restoration. The study results are in accordance with the report showing status of latencies and amplitudes after restoration of hearing in completely deaf children with the help of a cochlear implant[6].

Many animal researches with the goal of providing insight into possible mechanisms of acclimatization and deprivation effects have proved that organisation of the central auditory system is altered due to hearing loss and it reorganises once the hearing has been restored[7].

The possible underlying mechanisms explaining this improvement include improvements in synaptic efficacy and possibly increased myelination[9]. The changes in waveform morphology possibly reflect increase in neural synchrony as well as strengthened neural connections[9].

Some authors postulate two theories as to how the brain is able to " reorganise". The first hypothesis is that we have a reserve of neuronal connections and those that were previously in a state of rest replace connections that are no longer active. The other likely possibility is, simply, that new connections are formed. These changes may be slow and require extensive time and training, or they may be extremely rapid and spontaneous[2].

It can further be suggested that auditory system does not mature without stimulation. Nonetheless, the auditory system retains its plasticity, independent of age ,during the period of deafness since the re-introduction of stimulation by the hearing aid resumes the normal maturational sequence. Cortical evoked potential studies conducted on deaf children before and after cochlear implant support the above explanation[6].

There may be difference in the degree of plasticity which may be due to differences in
the overall level of redundant connectivity within different systems

XII. Conclusion

There is a significant improvement in the latencies and amplitudes of waves after the restoration of hearing in auditory deprived individuals. This in turn points to the
capacity of the central auditory system to reorganise itself.
This type of neural plasticity has implications for hearing aid use, acclimatization,
and deprivation effects.

Further research along these lines within the auditory system and in animal models of sensory deprivation may elucidate the role of specific molecular factors in the plasticity of auditory system. A better understanding of the plastic changes in the central auditory system after sensory differentiation, sensory stimulation, and learning may contribute significantly to improvement in the rehabilitation of damaged
or lost auditory function and consequently to improved speech processing and production.

XIII. References

1) Musiek F, Berge B, CAPD: (Mostly management in Katz) et al, Central Auditory processing disorders,Mostly management. Baltimore/; Williams and Wilkins, 1998:15-31
2) Musiek et al, Pathways, The Hearing Journal
3) Scheich H: Auditory Cortex: Comparative aspects of maps and plasticity: Curr Opinion Neurobiol 1991;1:247-263
4) Ohl FW, Scheich H. Learning induced plasticity in animal and human auditory cortex. Curr Opin Neurobiol. 2005 Aug: 15(4):470-7. Review
5) Johnson KL, Nicol T, Zecker SG, Kraus N. Developmental plasticity in the human auditory brainstem. J Neurosci. 2008 Apr 9:28(15):4000-7
6) Ponton CW, Don M, Eggermont JJ, Waring MD, Kwong B, Masuda A. Auditory System plasticity in children after long periods of complete deafness.
7) Willott JF, Phsyiological Plasticity in the auditory system and its possible relevance to hearing aid use, deprivation effects and acclimatization: Ear Hear,1996 June;17(3 Suppl) ;665-775
8) Syka J, Plastic changes in the central auditory system after hearing loss, restoration of function and during learning : Physiol Rev,2002 Jul;82(3);601-36
9) Gordon KA, Papsin BC, Harrison RV. Activity-dependent developmental plasticity of the auditory brainstem in children who use cochlear implants.

Disparities in Perceptions Of Healthcare Provider Communication Among Women: Findings From The 2003 Health Information Trends Survey (HINTS)

GJMR Classification (FOR)
321215,321214,321217,321216,321208

Ray Marks, EdD[1, 2] Haean Ok, EdD[2,3], John P. Allegrante, PhD[2,4]

Abstract-We analyzed perceptions regarding the frequency with which healthcare providers performed five communication tasks during clinical visits among a national sample of 3,848 women who responded to the 2003 Health Information National Trends Survey (HINTS). Results revealed that, regardless of racial or other subgroup or primary-provider status, providers failed to *always* perform the communication tasks. Being Hispanic, unemployed, uninsured, and young were among the variables associated ($P \leq .001$) with significantly lower perceptions that providers consistently performed the communication tasks.

Keywords: Disparities, health communication, patient-provider interactions, racial minorities, women

I. Introduction

Effective and consistent patient-provider communication is an important determinant of patient satisfaction, adherence to prescribed regimens, and health outcomes.1, 2 However, there are few data on how consistently healthcare providers perform communication tasks such as listening, explaining, being respectful, spending time, or including patients in joint decision-making, or the extent to which perceptions about whether providers perform such tasks vary by demographic group, health status, or insurance status. Provider communication in the context of cancer treatment and its prevention among minority women is especially important. Hispanic women, for example, are less likely than non-Hispanic women to utilize cervical cancer screening and have reported that receiving information, as well as having a supportive physician who communicates, reduces the barriers they perceive about getting screened.3 We examined the extent to which women perceived that five communication tasks were performed by their healthcare providers

About[1]-Department of Health and Physical Education and Gerontological Services, York College, City University of New York,
About[2]-Department of Health and Behavior Studies, Teachers College, Columbia University,
About[3]-Department of Sports Industry and Science, Mokwon University, Daejeon City, South Korea, and Department of Health
(Add Behavior Studies, Teachers College, Columbia University, 525 West 120th Street, Box 114, New York, NY 10027. Tel. (212) 678-3960, Fax (212) 678-8259. e-mail: jpa1@columbia.edu)
About[4]-Department of Sociomedical Sciences, Mailman School of Public Health, Columbia University.

II. Methods

Data from the 2003 Health Information National Trends Survey (HINTS)4, a public dataset of the National Cancer Institute, were used in this study. The data were collected between October 2002 and April 2003, and contain responses of 6,149 respondents who were 18 years and older from a probability sample of US households identified through random-digit dialing. Telephone interviews were conducted by trained interviewers using a standard interview schedule. The interview protocol includes constructs from established health communication theory.5

We analyzed the data from 3,848 women who responded to question HC-4, which asked how frequently the respondent's primary care provider had performed the following communication tasks in the past 12 months: 1) listening carefully, 2) explaining things, 3) showing respect, 4) spending enough time, and 5) involving the patient in joint decision-making. Responses to the question were recorded on a 4-point scale of frequency, where 4 = always, 3 = usually, 2 = sometimes, and 1 = never.

Demographic characteristics of respondents included: age in years (18-34, 35-64, and 65>); race (Hispanic, non-Hispanic white, non-Hispanic black, other or multiple races); education (<high school, high school, some college, college graduate); marital status (married, divorced, widowed or separated, never married, unmarried couple); employment status (employed, out of work, retired/student/homemaker, unable to work); and income (<$35,000, $35,000->$75 ,000, $75,000>). Other characteristics included: health status (excellent/very good, good, fair/poor); symptoms of depression during the past 12 months, coded on a 4-point Likert scale, recoded as follows: 1-1.25 (low) 1.26-1.66, 1.67-2.16, 2.17> (high); and health insurance coverage and access to a usual provider.

We analyzed the data by selected demographic, health status, and other variables using SUDAAN, a statistical analysis program designed specifically for analyzing complex datasets.6

III. Results

None of the racial groups perceived that healthcare providers *always* performed the five communication tasks (Table 1). Hispanic women, however, reported a lower mean rating of 3.35 $\pm$ .70 compared to non-Hispanics who

reported scores of 3.45-3.48. Moreover, Hispanic women not only rated they providers as being inconsistent in always performing the communication tasks, they also reported higher rates of never receiving the same communication opportunities than other women (P = .000 for all five communication tasks), especially being involved in joint decision-making (Table 2).

Ratings also varied by age, work status, health status, level of reported depression, and insurance status (P < .001). Women age 18-24 years reported lower composite ratings (3.41 + .65) than those over age 65 years of age who reported an average rating of 3.58 + .55 (P = .001). Being18-34 was also associated with a higher frequency of responses in the never category for all five communication tasks. Being out of work yielded the lowest perceived rating that the five communication tasks were always performed, followed by being unable to work (P = .018). Finally, reporting excellent, very good or good health versus fair or poor health, being less depressed, and having insurance were all associated with higher overall ratings about providers always performing the five communication tasks (P < .001).

IV. Discussion

Regardless of racial group, health care providers failed to consistently perform five important communication tasks. However, consistent with one previous report,7 women who are Hispanic, unemployed, uninsured, and young had significantly lower perceptions than non-Hispanic, employed, insured, and older women regarding the consistency with which healthcare providers performed the communication tasks.

Women who perceive that their provider is less than responsive or spends too little time communicating with them are likely to feel lower satisfaction levels and lower motivation levels to carry out recommended advice. Moreover, women who need the most counseling, i.e., those who report being in poor health or depressed, appear to be receiving less communication than they would desire.

These results suggest that healthcare providers need to improve their communication with Hispanic women who are young, uninsured, and unemployed, and those already in poor health or depressed. Examining why these discrepancies exist and what can be done to improve patient-provider communication should be investigated further.

V. References

1) Betancourt JR, Green AR, Carrillo JE. The challenges of cross-cultural healthcare--diversity, ethics, and the medical encounter. Bioethics Forum. 2000; 16:27-32

2) Freimuth VS. The chronically uninformed: Closing the knowledge gap in health. In: Ray EB, Donohew L, eds. Communication and Health: Systems and Applications. Hillsdale, NJ: Erlbaum; 1990: 212-237.

3) Byrd TL, Chavez R, Wilson KM. Barriers and facilitators of cervical cancer screening among Hispanic women. Ethn Dis. 2007; 17:129-134.

4) HINTS—Health Information National Trends Survey. Main Study Interview Instrument (English). Post-Analysis Version. National Cancer Institute; 2003.

5) Nelson DE, Kreps GL, Hesse BW, et al. The Health Information Trends Survey (HINTS): Development, design, and dissemination. J Health Commun. 2004; 9:443-460.

6) SUDAAN. Available at:http://www.rit.org/sudaan. Accessed September 27, 2006.

7) Rutten LJ, Augustson E, Wanke K. Factors associated with patients' perceptions of health care providers' communication behavior. J Health Commun. 2006; 11(Suppl 1):135-146.

TABLE 1—Composite Ratings of Healthcare Provider Communication Tasks, by Race (N = 3,308)

	N	Mean	Std. Deviation	Std. Error	95% CI		Minimum	Maximum
Race					Lower Bound	Upper Bound		
Hispanic*	384	3.35	.70	.03610	3.2803	3.4223	1.00	4.00
Non-Hispanic white	2,363	3.48	.59	.01230	3.4621	3.5104	1.00	4.00
Non-Hispanic black	415	3.46	.67	.03321	3.3950	3.5255	1.20	4.00
Other or multiple race	146	3.45	.63	.05281	3.3470	3.5558	1.25	4.00
All races	3,308	3.46	.62	.01086	3.4445	3.4871	1.00	4.00

Note. CI = confidence interval. Response scale: 1 = never, 2 = sometimes, 3 = usually, 4 = always.

*P = .001 (df = 3, F = 5.201).

TABLE 2—Percentage Distribution of Respondents Reporting on the Extent to Which Healthcare Providers Performed Five Communication Tasks in the Past 12 Months, by Race (N = 3,308)

	% Responding Never			% Responding Always		
Communication Task	Hispanic	White	Black	Hispanic	White	Black
Listening	3.7	0.9	2.2	62.1	60.4	66.3
Explaining	2.9	1	1.4	57	62.6	66.7
Being respectful	1.6	0.9	1.4	70.3	71.5	75.4
Spending enough time	5.2	2.8	5.1	51.7	53.7	57.1
Mutual decision-making	7.7	2.7	4.1	60.2	62.7	64.9

Note. All white and black respondents are of non-Hispanic origin.

$P < .05$

Kernel Based Off-Target Analysis of Rnai Experiments

GJMR Classification (FOR) 320304,321011

Karol Kozak[1] Gabor Csucs[2]

***Abstract*- The occurrence of off-target effects is still a challenging aspect in the interpretation of data from large-scale RNA interference screens. Up to now many methods have been found to reduce these effects, but they cannot completely avoid them. In the framework of this manuscript a tool for the analysis of off-target effects is developed which can be applied after high content screenings. This tool provides new variant based on kernel method to search for similarities between the siRNAs and the genes. The method is based on Sequence Alignment Kernel, a function reflecting the quantitative measure of match between two sequences. These kernels measure sequence similarity based on shared occurrences of length subsequences, counted with up to mismatches, and do not rely on any generative model for the positive training sequences.**

Keywords-bioinformatics, pattern recognition, kernel function, sequencing.

I. Introduction

RNA interference (RNAi) is a powerful method for post-transcriptional silencing of specific genes [1]. It is triggered by short interfering RNAs (siRNAs), ~21 nt long, which bind to sequence-identical mRNAs causing their degradation and consequentially a decrease of their protein level. In current gene function studies this siRNA-mediated mechanism plays an important role e.g. for future therapeutic purposes [2]. One functional genomics application of RNAi is the High Content Screening (HCS) technology [3] which uses images of cells to detect the phenotypical effect of a treatment with chemical compounds or an siRNA transfection. This technology allows a very large number of RNAi experiments in parallel and therefore facilitates the complex analysis of gene functions. A problem, however, one has to deal with in RNAi screens are off-target effects. They are defined as the occurrence of non-specific effects caused by the transfection of siRNA, e.g. unintended knockdowns of other genes than the target one. Although ongoing research efforts try to determine the causes for these effects, the mechanism is still not fully understood. Some indicators for off-target effects have been detected, but unfortunately, they do not explain the whole underlying machinery, which makes a prediction of these effects almost impossible so far. Many companies provide siRNA libraries and apply a number of methods or algorithms to optimize the siRNA design, but nevertheless Off-target effects cannot be completely avoided. So the Following questions arise: How to deal with the unintended off-target effects cannot be completely avoided. So the following questions arise: How to deal with the unintended Gene silencing occurring in RNAi screens? How much do off-target effects influence the interpretation of the screening results and do some false positives show up due to these effects?

In this paper we describe a kernel based methods, which guides through the analysis of potential off-target effects for every siRNA of interest. Furthermore, it should support the verification of top hits in the RNAi screen or the determination of false positives, which can be excluded from the results afterwards. Such an analysis should help to better understand how unintended gene silencing is affecting the results of the screen and hence, to minimize its influence on the data interpretation.

II. Off-Target Effects In Rnai Screens

Since the discovery of RNA interference as a new method for specific gene silencing, researchers have invested great efforts to optimize this technology for a perfect use in functional genomics studies. Nevertheless, the translation of RNAi into a research tool has its obstacles. The transfection of siRNA into the cell can lead to unintended side effects, e.g. the downregulation of several non-targeted genes. These unintended phenomena are called off-target effects. As they can cause measurable phenotypes, it is a critical problem one has to deal with when interpreting screening data. In the worst case even the top hits of an RNAi screen can result from these effects [4]. Therefore it is of great importance to understand the mechanism behind off-targeting so that strategies can be developed to minimize their effects. Off-target effects can be caused by many different mechanisms: lipid-mediated response, interferon response: (through long dsRNA, through high siRNA concentration), impact on cell viability, incorporation of the sense strand, siRNA-sequence-dependent off-target effects. Fedorov and colleagues have demonstrated that cationic lipids, which are typically used to deliver siRNA, can induce broad changes in gene expression profiles [5]. Furthermore, it has been shown that dsRNAs longer than 23 bp can induce an interferon response in a cell type-specific manner [6, 7]. This antiviral interferon pathway machinery can also be mediated due to a high siRNA concentration [8]. Current studies have reported that an siRNA concentration of 20nM used in RNAi experiments reduces the nonspecific effects on gene expression. All these unintended effects can easily be eliminated by using optimal lipid and siRNA

About[1]-Large Scale Data Handling, LMC, ETH Zurich (telephone: +41446333494 email: karol.kozak@lmc.bio.ethz.ch)
About[2]- author Head of Light Microscopy Center, LMC, ETH Zurich (telephone: +41446333494 email: csucs@lmc.biol.ethz.ch)

concentrations and adopting stringent siRNA design filters, e.g. transfecting only siRNA with a length of 21 nucleotides. siRNA delivery also influences the cell viability and can result into toxic phenotypes. Federov et al. have revealed a strong correlation between toxicity and the presence of a 4 bp motif (UGGC) in the toxic siRNA strand [9]. However, chemical modifications of the siRNA not only reduce the amount of off-target effects in general [10], they also inhibit the toxicity of the siRNA-mediated RNAi [9]. As a further aspect off-target effects can be caused by the incorporation of the sense siRNA strand into the RISC complex instead of the antisense strand (iv). This can also be prevented by applying some modification [11, 12]. All unwanted effects mentioned above are avoidable by reducing the concentrations, applying some modifications and using correct reagents in the experiment. But this is not sufficient to eliminate off-target effects completely. The specificity of the siRNA sequence is the crucial factor in an RNAi experiment [13]. Gene expression silencing through the RNAi machinery works perfectly if the siRNA is totally complementary to its target mRNA. Several publications have shown that even single nucleotide mismatches between the siRNA and the target mRNA greatly decrease the rate of mRNA degradation [14, 15]. However, also partial complementarily between siRNA and mRNA seems to be sufficient to reduce the number of transcripts [16]. Based on this tolerance for mismatches and gaps in base pairing with targets, siRNAs could have up to hundreds of potential target sequences in the genome. But the degree of complementarily between the two sequences needed for silencing is still unclear. Many research groups try to detect the mechanism behind the sequence-dependent off-target effects and some parts of this puzzle have already been identified. First of all, it has been reported that off-target effects occur with a high probability, if the siRNA shows a nearly exact homology region to an off-target gene [11, 17]. With nearly exact the authors mean a complementarily of ~90%, e.g. 17 nucleotides out of 19 match. However, Saxena et al. observed that 3-4 mismatches between siRNA and mRNA can still silence a target gene but by triggering translational repression instead of mRNA degradation [18]. Therefore one can find cases where a 21-nucleotide double-stranded RNA sharing only partial complementarily with the target mRNA is still competent to cause gene silencing via translational repression. So the question is how many and at which position mismatches are tolerated to knockdown unintended genes. Jackson and colleagues have reported a reduction in the level of mRNA transcripts, if the siRNA shares as few as 11 contiguous nucleotides or a total of 15 nucleotides with the mRNA [11]. Other studies revealed that off-target gene silencing is caused by a 7 nt motif, but this also depends on the characteristics of the mRNA, including the sequence context surrounding the complementary region e.g. its position and copy number in the mRNA [4]. Further analyses show a high tolerance for mismatches outside of the first 2–8 bases of the antisense siRNA-strand whereas differences within this 5' end of the siRNA are not tolerated [19]. The region ranging from position 2-7 of the antisense strand is called the seed region (see figure 1) and seed region complementarily seems to play a major role in the occurrences of off-target effects [16].

Fig. 1: Structure of an siRNA: 21 bp RNA duplex with 2 nucleotide 3'overhang on each strand, containing a seed region and a cleavage site.

III. Methods

A. *Concept for a bioinformatics analysis of off-target effects*

Available sequence analysis tools fail to predict off-target effects for siRNA sequences. Building upon current understanding for the occurrence of off-target effects a new modular analytic process is introduced in this paper. This process can be specifically adopted to a variety of analysis methods to identify potential off-target genes for every siRNA of interest. In the first subsection three different scenarios for a meaningful application of such an off-target analysis are elaborated.

To verify the results of an RNAi screen normally four separate siRNA-oligos are used to silence the same mRNA at different positions. If all four oligos show an effect of similar magnitude, one can be reasonably sure that the down regulation of the intended target gene had worked. This gene which might play an important role in the examined pathway is called a top hit. If the whole RNAi machinery would work perfectly, the results would only include top hits and genes where none of the 4 oligos show an effect. But unfortunately, this is not the case. A large number of unexpected phenomena occur in the screening results which can arise from many different causes:

Effect in one oligo out of four: In the first scenario only one out of the four oligos shows an effect in the RNAi screen. This could be due to an off-target effect, which means that there exists a homology region between this siRNA and an unintended gene. This unintended gene could actually be a top hit of the screen, which would explain the observed effect. By performing the off-target analysis one can determine whether there exists such a homology with a top hit or not.

Effect in two oligos out of four: Quite often only two out of the four oligos show an effect in a screen. This again could have been caused by a complementarily between the siRNAs and one or two different nontarget genes. With the off-target analysis it could be determined if such a homology, e.g. with a top hit, exists. As an alternative these two siRNAs might knockdown the target gene, whereas the other two siRNAs just did not work. Hence, the target gene could play an important role in the examined pathway and is worth a second look.

B. *The analytic process*

The main idea is to identify potential off-target effects due to sequence homology regions between siRNAs and mRNAs. For flexibility and extensibility reasons it is composed as a set of process steps, which must be performed in sequence to get to an effective analysis. In figure 2 the complete concept is schematically shown, each box representing one part of the analytic process.

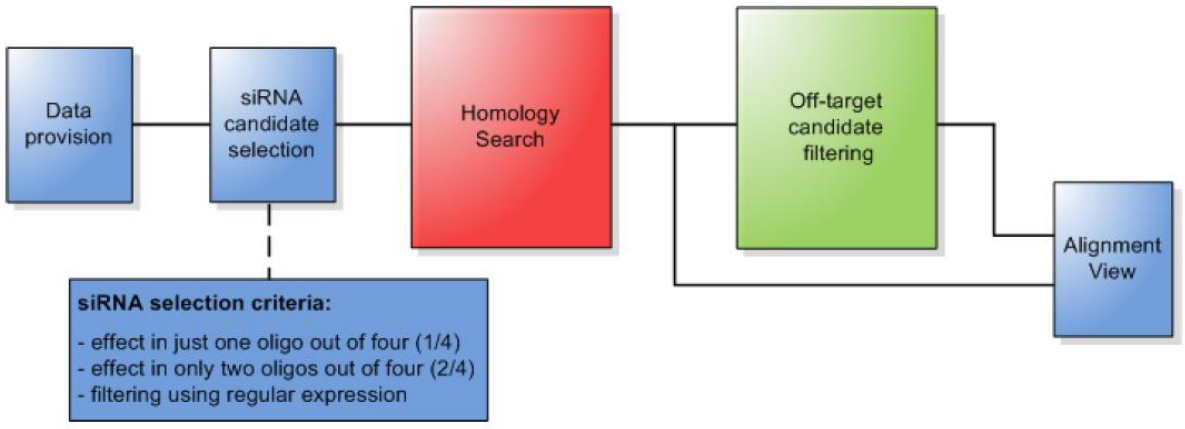


Fig.3. General Structure of the concept for analyzing screening results of off-target effects.

In the first step the data needed for the off-target analysis is supplied. Since the analysis is based on sequence-dependent interactions between siRNA and mRNA, the sequence information for every siRNA has to be given. Depending on the application scenario the results of the RNAi screen including hits must also be available. This data should be displayed in a table format, provided for example by the companies which designed the siRNAs. As an alternative this table can also be generated after the experiment containing its evaluated hit results. In a second step, it is important to select the siRNA candidates for which the analysis should be performed. This is necessary to reduce the amount of examined siRNAs. Otherwise the runtime for the homology search performed in the next step would be too long and the output of the analysis would not be manageable anymore. Therefore, depending on the purpose of the analysis, it has to be decided which siRNAs should be tested for off-target effects.

The following selection options are derived from the above application scenarios.

• The occurrence of an effect for just one siRNA out of the four oligos is a strong hint that an off-target effect has appeared. So one option is to select all these single siRNAs which show an effect and analyse their possible off-target properties.

• In an RNAi experiment it is also quite common that two out of the four oligos behave completely different. Therefore these two siRNAs which show an effect can be analysed for their potential off-target genes.

After selecting the siRNAs which should be analysed for potential off-target genes, the next step is to find homologies between these siRNAs and all mRNAs. This concept contains many variants for such a homology search using different algorithms to perform a sequence alignment between siRNA and mRNA. A detailed description of the different homology search strategies is given in the next subsection.

The resulting list of a homology search can be too long to find the important results just by taking a look at it. Therefore the next step is to filter this list to reduce its size to meaningful results. Additionally, it is possible to take a look at any specific alignment at any time. The alignment view includes, beside the alignment itself, all necessary information of the two sequences. It should be designed in such a way that a comparison between different alignments is feasible.

C. *Kernel method in homology search*

In this analysis step of off-target effects it can be determined if there exists a homology region between the selected siRNA sequences and the mRNAs. Many different sequence alignment algorithms to perform such a homology search are available, but they are not optimal for the purpose of this process step by default. Therefore, three different strategies for the use of these algorithms have been developed to find nearly exact homology regions as well as small local complementarities (see also figure 3).

Kernel method

The Basic Local Alignment Search Tool (BLAST) [25] is one of the most popular algorithms for homology search and can be applied to find nearly identical gene regions for a specific siRNA sequence. Unfortunately, the BLAST algorithm is not applicable to find all potential off-target candidates, because genes with only partial complementarily are missing. Despite this disadvantage, BLAST is an effective tool to find out immediately if obvious off-target genes exist with a nearly identical nucleotide sequence to

the siRNA. Therefore the Kernel base homology search is the first strategy for a homology search in this concept.

In this paper we make use of Sequence Alignment Kernel (Watkins, 2000; Surkov *et al*., 2001) to define the measure of similarity between two oligo sequences. Our method is based on building the *kernel function* $K(s_i, s_j)$ as a quantitative measure of similarity between two target gene mRNA of observer siRNA sequences *R* and *Q*. Suppose we are given a matrix Swap*(a, b)* which defines the score corresponding to a single point mutation of letter *a* into letter *b* or vice versa (the matrix is symmetric). We are also given a vector Gap*(a)* which defines the score corresponding to a single point deletion or insertion of letter *a*. One of the schemes for simultaneous generation of two sequences over a given alphabet was proposed by Watkins (2000) [15]. The generative model may emit either two letters (one into each sequence), only one letter into the first sequence (which corresponds to a gap into the second one), or only one letter into the second sequence (which corresponds to a gap into the first one). The model is completely defined by the probabilities for each pair it may emit. For any two nonempty sequences there are several ways (or paths) to generate them using this model. For every such path the corresponding probability is the product of probabilities along the path. The total probability *P (a, b)* that the sequences *a* and *b* will be generated by the model is the sum of probabilities of all the paths that lead to generating the given pair. Watkins (2000) has proven that the function *P (a, b)* is symmetric and positively definite, and so may be used as a kernel for kernel-based algorithms.

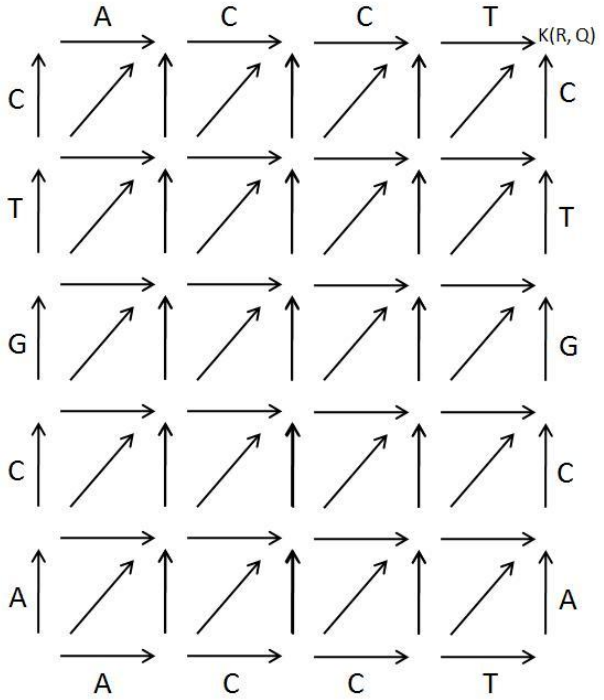


Fig.4. Version of sequence alignment kernel.

Suppose we are given two sequences to align, *Q* = '*ACCT* ' and *R* = '*ACGT C*'. Let us write them along the two dimensions of an empty matrix (Fig. 4). In each cell $p_{i,j}$ of the matrix we will be keeping the probability that$Q_1 \ldots j$ aligns with $R_1 \ldots i$. It is convenient to start the calculations from the bottom left corner, which is initialized with the value of 1. Then, we fill all the other cells using the recursive formula:

$$p_{0,0} = 1,\ p_{i,0} = p_{0,j} = 0, \text{ for } i > 0 \text{ and } j > 0,$$
$$p_{i,j} \leftarrow \text{Swap}(R_i, Q_j) \cdot p_{i-1,j-1} + \text{Gap}(Q_j) \cdot p_{i,j-1} + \text{Gap}(R_i) \cdot p_{i-1,j} \quad (17)$$

Where the Swap(a, b) matrix and the Gap(a) vector of probabilities are given as parameters to the algorithm. The kernel value we are looking for is the probability

$$K = p_{|R|,|Q|} \quad (18)$$

in the top right corner of the matrix. Note, that to calculate values on any 'backslash' diagonals

Of the *p* matrix ($i + j = D$) we only need to know the values on the two preceding diagonals: $i + j = D - 1$ and $i + j = D - 2$.

The problem with this type of algorithms is their computational complexity, which makes them very slow in real applications. Therefore their use with the whole mRNA database is not possible. The developed concept offers, beside the Kernel method search, two different alternatives of building a local alignment without getting into the mentioned runtime problem.

D. Smith-Waterman algorithm

The Smith-Waterman algorithm is an accurate algorithm used to build local alignments between two sequences [26]. Since its use with all mRNAs from the database is not practicable, a feasible alternative is to limit the number of mRNAs to approximately 200. By reducing the number of sequences it is possible to perform a local alignment for all the siRNAs. The subset of mRNAs could contain all genes which seem to play a role in the explored cell pathway, e.g. the top hits of the screening experiment. Finding a homology between an siRNA and a top hit gene could explain the unexpected phenomenon observed in the RNAi screen.

E. Seed-Motif-Search combined with the Smith-Waterman algorithm

Because of the mentioned runtime problem when performing a local alignment with the Smith-

Waterman algorithm, a third variant to search for homology is introduced here. In this variant an initial step reduces the length of the mRNA sequences to enable the use of a local alignment algorithm. This reduction is made because the seed region of the siRNA seems to play a significant role in causing off-target effects. At the beginning all occurrences of the seed motif of every siRNA are localized in the genes (see figure 3). After detecting this small region, a sequence of ~50 nt around this seed motif is cut out in the mRNA. Thus, as a result of this first step, a huge number of

sequences of ~50 nt in length are obtained containing the seed region of each siRNA. Due to the small length of the sequences it is now possible to perform a local alignment with the Smith-Waterman algorithm. The disadvantage of the Seed-Motif-Search is that it limits the results to those genes which perfectly match with the seed region of the siRNA. This limitation, however, seems to be a good starting point for the analysis of off-target effects.

F. Filter options

The result of the homology search is a list of potential off-target candidates. This list can be very large including many false positives. To reduce its size to get only the most probable off-target candidates and also to make the list manageable, a great number of filtering options are provided. Beside the possibility to filter for screening top hits, the results of the homology search can be further tested for the potential causes of off-target effects that are listed in table 1. The following filter options present a central part of the analysis concept (see also figure 5):

• Threshold filter: Only alignments which are greater or smaller than a specific threshold are shown. This threshold could be for example the alignment score or the E-value in the BLAST output.

• Position of matches in the alignment: The positions at which the alignment should contain a match can be specified with this filter. It can be used, for example, to show only alignments matching at position 9-11 in the siRNA sequence, because this central region seems to play an important role in the occurrence of off-target effects. Optionally also a G:U wobble can be tolerated as a match in the alignment.

• Number of matches in the alignment: The total number of matches which should at least occur in the alignment is defined in this filter.

• Length of a continuous match in the alignment: With this filter the length of a continuous match can be determined, e.g. the occurrence of a stretch of at least 11 bases in the alignment.

• Location within the mRNA: The location of the alignment within the mRNA is specified in this filter. It can be defined if the siRNA should match in the 5'UTR, the CDS and/or the 3'UTR of the mRNA.

• Multiple homology regions of an siRNA in one mRNA: Only the results of an siRNA which has multiple homology regions in the same mRNA are shown.

• Strand selection: This filter extracts complementarities between the gene and either the sense or the antisense strand of the siRNA.

• Specific gene or a list of genes: The list of off-target candidates can be filtered for one or more genes of interest. Such genes could be for example the known top hits of the screen.

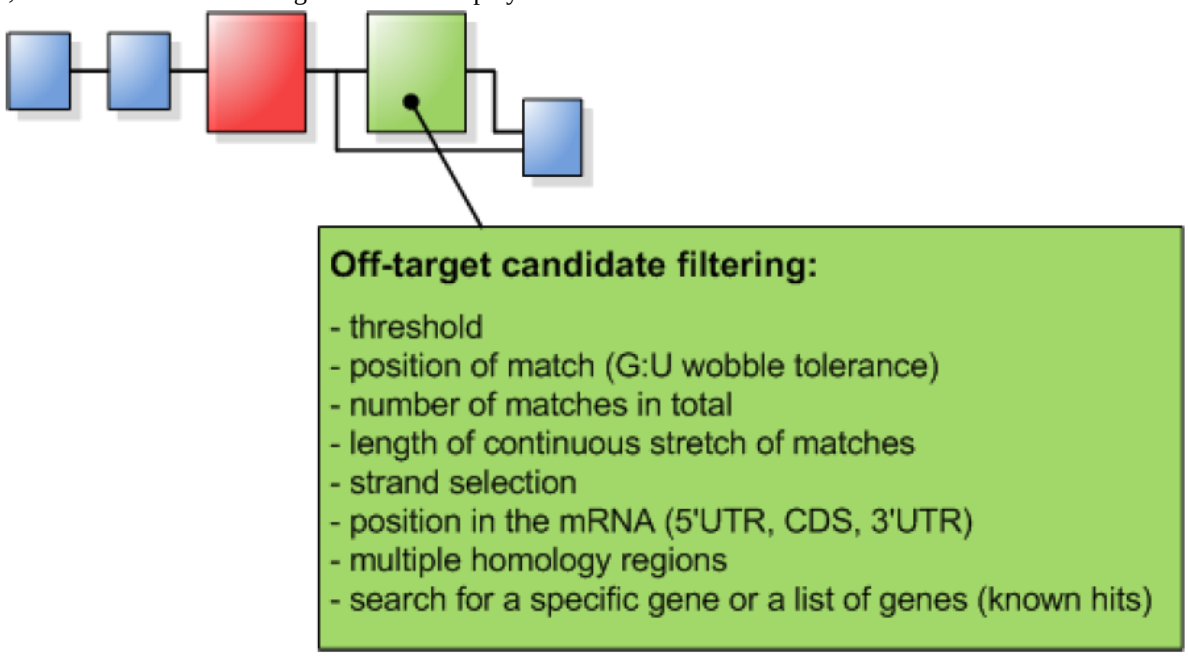


Fig.5. Filtering of off-target candidates through different options.

IV. Conclusion

The nodes described above in combination with the existing nodes provided from HCDC and KNIME represent the toolkit for creating individual workflows. To demonstrate how an analysis of off-target effects can be performed, an exemplary workflow is shown in figure 6. In a first step the data containing the siRNA information and optional the screening results are read in. This can be realised by using an already existing I/O node from KNIME, the File Reader. With this node it is very simple to upload a table in .csv format. Afterwards, the user can take the siRNA Selection Node and connect it with the File Reader. Since the siRNA Selection Node is quite flexible concerning the column headers, normally no adjustments of the table properties are necessary in advance. After the siRNAs to be analysed have been chosen, the homology search is performed in the next step. This can be done in two different ways. To look for nearly exact complementarities the BLAST Node can be appended in the workflow, see variant A. As an alternative, one can also use the Seed-Motif-Search Node at this point to find small local homology regions, see variant B.

After the BLAST search the results can be filtered by using different filter nodes of this software. In figure 5, for example, first the Alignment Filter is used to get only alignments containing 17 matches. Afterwards, the Threshold Filter removes the target genes from the input list by using an upper boundary. At the end of the workflow the Alignment View Node is appended to take a more detailed look at the filtered results.

In variant B, the user can also perform an arbitrary number of filtering steps, but it is recommended to sort the outcome of the homology search before doing so. The output table of the Seed-Motif-Search Node is sorted by the mRNAs occurring in the genbank file. As in variant A filter nodes can be individually used to reduce the size of the output table afterwards. Finally also the Alignment View Node can be added at the end of the workflow. These two variants demonstrate the general structure of a workflow to perform an off-target analysis of RNAi screening data. The filtering steps are of great importance in the analytic process, because they help to remove false positives from the output table and to find the most probable off-target genes. As mentioned before, the filter nodes can be used and combined completely flexible. This is demonstrated in figure 6 showing three sample workflows for performing meaningful filtering.

It has been reported that siRNAs can cause off-target effects because of their miRNA-like behavior [21]. This means that there exists a seed region complementarity with the 3'UTR of the mRNA. The effect could be amplified if this homology region occurs multiple times in the 3' end of the mRNA. To filter for these off-target effects the following filter nodes shown in variant A of figure 6 can be connected. The Alignment Filter gets the results which show a stretch of matches at the seed region (positions 13-18).

Afterwards the Location Filter reduces the table to those alignments located in the 3'UTR of the mRNA and finally the Multiple Hit Filter shows only the results where an siRNA has at least 2 homology regions in an mRNA. This example demonstrates the advantage of the workflow environment. The filtered output tables are always available for each node enabling the user to compare the results after each filtering step. Variant B in figure 6 shows that also combinations of the same type of filtering node are allowed. The first Alignment Filter for example only displays the alignments which contain a match at the positions 9-11 and 13-18. Additionally, the second Alignment Filter node reduces this output table to those alignments having at least 11 matches in total. As a last example, a very common filter combination to analyse off-target effects is shown in variant C of figure 6. Off-target effects triggered by a homology region with a top hit gene of the screen are normally of great interest. The occurrence of such off-target effects would explain unexpected phenomena in screening results. Therefore, the Text Filter node can be used with a list of top hit gene symbols so that only siRNA results showing a homology region with a top hit are included in the output table afterwards. However, not all of these results are correct off-target candidates and further filtering nodes have to be applied to get the most probable ones.

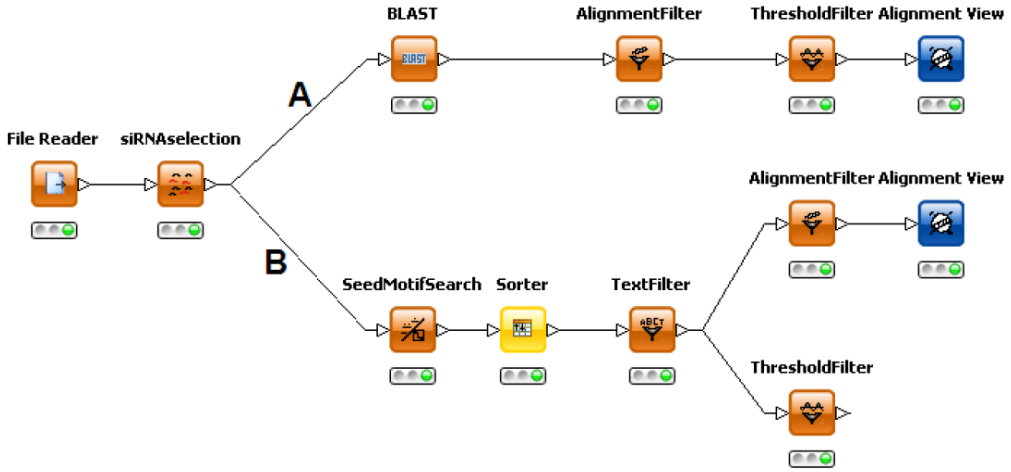


Fig.6. Workflow for analyzing off-target effects.

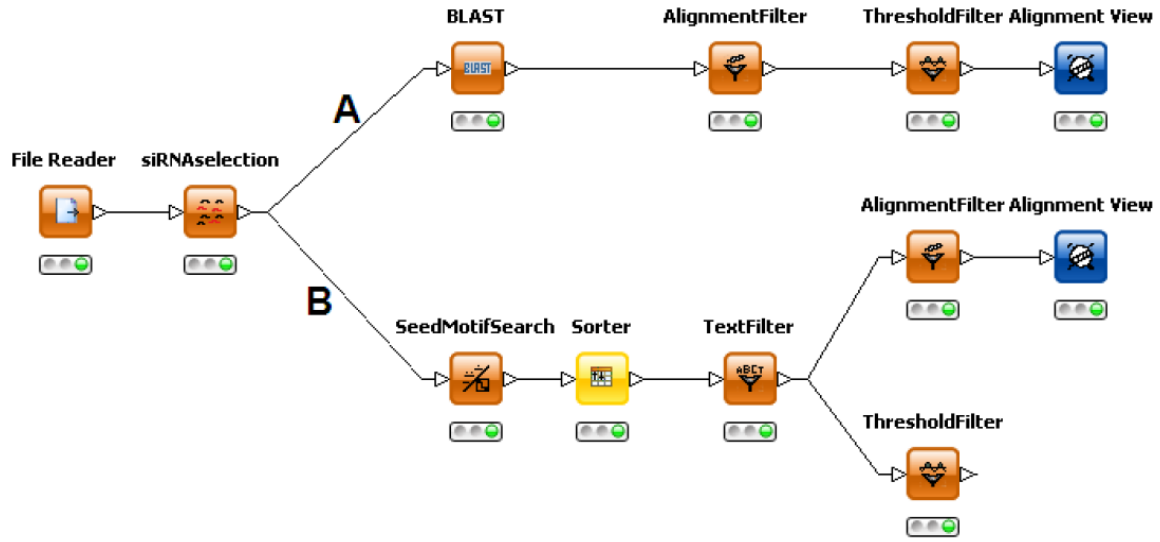


Fig.7. Filter combinations in a workflow to analyse off-target effects.

V. Experimental Data

For the purpose of validating the new toolkit and showing its usability in actual research projects it has been applied to experimental data from High Content Screenings. The data came from an RNAi screen to probe for host cell factors important for the Salmonella invasion. Essential for the pathogenesis by Salmonella is its ability to invade epithelial cells [27]. A specific assays used in this screen enables the visual detection of Salmonella in the host cell, step 5 of the invasion. In this project 7000 genes in total are targeted with a first siRNA library in an RNAi screen. Afterwards 300 hits including some educated guesses are grouped as a second library, which is tested again in a screen. As in the first dataset the result of this analysis is a so-called hit rate for each siRNA. This hit rate is calculated by the logarithm to the basis 2 of a normal ised percentage of infected cells. The results of the small RNAi screen with 300 genes are the second dataset used for off-target analysis in this paper.

G. Effect in one out of four oligos

For a better sense of the result interpretation, a so-called control set is adopted, which includes a number of oligos showing no effect in the RNAi screen. By performing the same homology search and filtering workflow the results of the test and the control set can be compared, for example with respect to the number of potential off-target candidates.

For comparison reasons the same workflow is also applied to a control set extracted from the salmonella dataset (see figure 8). All 8 oligos, coming from 2 different genes, have a hit rate between -0.2 and 0.18, which means that they do not show any phenotypical change in the screen.

The result of the homology search with the center region plus the alignment and threshold filtering is shown in figure 9. For 6 out of the 8 oligos off-target candidates are found with quite a high alignment score. Unfortunately none of the resulting genes is included in the provided dataset, which is a handicap in the further interpretation. The size of the dataset with only 300 genes is a constraint compared to the first dataset with more than 20,000 entries. In general no significant difference in the number of entries in the output table seems to exist between the test and the control set when applying the same analysis workflow. However, it would be incorrect to infer the reliability of the listed off-target candidates from these first experiments.

Gene_ID	N...	NCBI_Gene_Description	Sequence
64710	NUCKS1	nuclear casein kinase and cyclin-dependent kinase substrate 1	AACCGGAAAGCCCGCCAGAAA
64710	NUCKS1	nuclear casein kinase and cyclin-dependent kinase substrate 1	AGGAAGGTTGTTGATTACTCA
64710	NUCKS1	nuclear casein kinase and cyclin-dependent kinase substrate 1	AAGAACCTACTTAAGATAGAA
64710	NUCKS1	nuclear casein kinase and cyclin-dependent kinase substrate 1	TCGGGCCCTCCCACTAAGAAA
28964	GIT1	G protein-coupled receptor kinase interactor 1	CCGGATCACAAGAATGGGCAT
28964	GIT1	G protein-coupled receptor kinase interactor 1	CAGCCTTGACTTATCCGAATT
28964	GIT1	G protein-coupled receptor kinase interactor 1	GCCGCTGAGGATGTCCCGAAA
28964	GIT1	G protein-coupled receptor kinase interactor 1	CACCTTGATCATCGACATTCT

Fig.8. Control set of the salmonella dataset: 8 oligos with no effect in the screen

N...	siRNA...	S...	SubjectN...	SubjectDesc	D	S ..	Subj...
NUCKS1	TCGGGCC...	84445	NM_032429	Homo sapiens leucine zipper, putative tumor suppressor 2 (LZTS2),mRNA.	76	3-19	868-884
NUCKS1	TCGGGCC...	3796	NM_004520	Homo sapiens kinesin heavy chain member 2A (KIF2A), transcriptvariant 1, mRNA.	70	2-15	34-47
NUCKS1	TCGGGCC...	3796	NM_001098511	Homo sapiens kinesin heavy chain member 2A (KIF2A), transcriptvariant 2, mRNA.	70	2-15	34-47
NUCKS1	TCGGGCC...	127833	NM_177402	Homo sapiens synaptotagmin II (SYT2), transcript variant 1, mRNA.	66	1-15	7126-7140
NUCKS1	TCGGGCC...	127833	NM_001136504	Homo sapiens synaptotagmin II (SYT2), transcript variant 2, mRNA.	66	1-15	7136-7150
GIT1	GCCGCTG...	54901	NM_017774	Homo sapiens CDK5 regulatory subunit associated protein 1-like 1(CDKAL1), mRNA.	70	3-16	1844-1857
GIT1	CCGGATC...	846	NM_000388	Homo sapiens calcium-sensing receptor (CASR), mRNA.	66	1-15	4223-4237
GIT1	CAGCCTT...	28964	NM_001085454	Homo sapiens G protein-coupled receptor kinase interacting ArfGAP 1(GIT1), transcript variant 1, mRNA.	90	2-19	1001-1018
NUCKS1	AGGAAGG...	10584	NM_006438	Homo sapiens collectin sub-family member 10 (C-type lectin)(COLEC10), mRNA.	75	1-15	1306-1320
NUCKS1	AGGAAGG...	23479	NM_213595	Homo sapiens iron-sulfur cluster scaffold homolog (E. coli) (ISCU),nuclear gene encoding mitochondrial protein, transcript variant 2...	68	1-19	214-232
NUCKS1	AGGAAGG...	4112	NM_177404	Homo sapiens melanoma antigen family B, 1 (MAGEB1), transcriptvariant 2, mRNA.	66	3-17	568-582
NUCKS1	AGGAAGG...	4112	NM_177415	Homo sapiens melanoma antigen family B, 1 (MAGEB1), transcriptvariant 3, mRNA.	66	3-17	642-656
NUCKS1	AGGAAGG...	4112	NM_002363	Homo sapiens melanoma antigen family B, 1 (MAGEB1), transcriptvariant 1, mRNA.	66	3-17	725-739
NUCKS1	AAGAACC...	2803	NM_002078	Homo sapiens golgi autoantigen, golgin subfamily a, 4 (GOLGA4),mRNA.	70	2-15	1179-1192

Fig.9. Second control set applied to the Seed-Motif-Search Node (motif position: 3-9), the Alignment Filter (matches at position 3-15) and the Threshold Filter (alignment score between 66-94) just because the number of results for the test and the control set are the same.

The oligos of the control set do not show any effect in the corresponding RNAi screen, which does not imply that there exist no off-target effects in general. The off-target candidates for the control set listed in figure 9 might also be correct without having an influence on the screening outcome. However, an off-target effect occurring between an oligo and a top hit of the screen would explain the divergent hit rates. To identify only this type of results with a homology between an siRNA and a top hit of the screen, the Text Filter has to be used as the first filtering node in further workflows. It is expected that such a significant off-target candidate explains the unexpected phenomenon occurring in the screening data and that it is not included in the results of the control set.

H. Analysis including the Text Filter

For provided dataset a list of genes with high confidence is provided by the research group. This is used in the following workflows to find homology regions between the selected siRNAs and a top hit. The corresponding graphical representation of the workflow is shown in Figure 10.

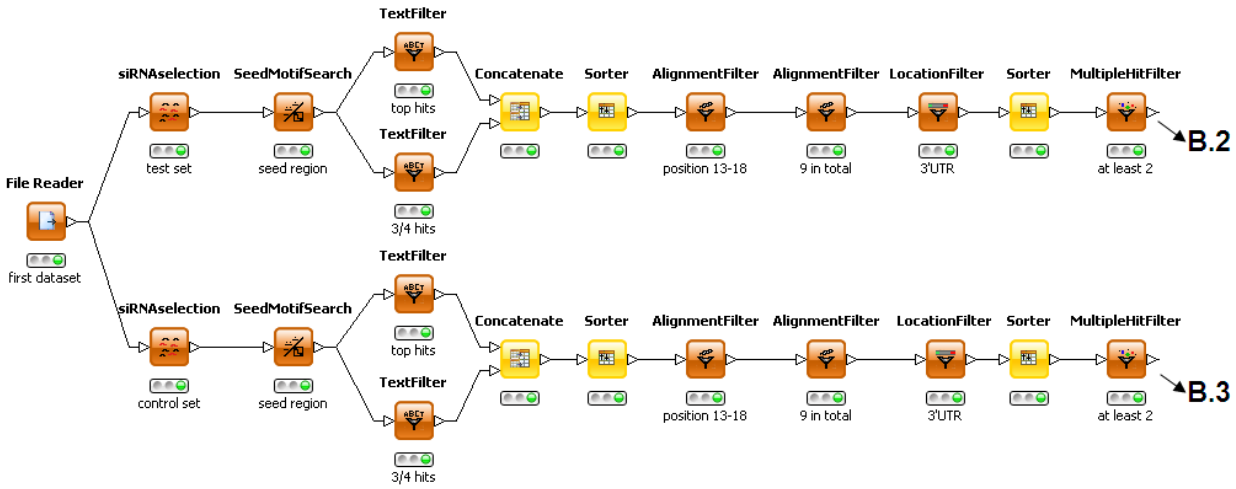


Fig.10. Workflow to analyse miRNA-like off-target effects with the first test and control set.

Initially, all oligos of the test and control set are applied to the Seed-Motif-Search Node with the default settings. Afterwards two Text Filter nodes are used in parallel. The first one filters for the above mentioned top hits by scanning the mRNA descriptions for one out of the 42 top hit gene symbols. The second Text Filter uses a list of 98 genes belonging to the 3/4 category where 3 oligos have a hit rate >0.5. These genes also have a high confidence level concerning their role in the salmonella assay. Subsequently these two result lists are concatenated and sorted. To further reduce their size a couple of filter nodes are applied in sequence. First an Alignment Filter is used to get only the results containing a match at position 13-18. The second Alignment Filter sets the constraint that the alignments must contain 9 matches in total. Additionally, the Location Filter shows only the alignments located in the 3'UTR of the mRNA and finally a Sorter in combination with a Multiple Hit Filter is applied to obtain genes occurring at least two times in sequence in the output table. The resulting list of this workflow contains the miRNA-like offtarget effects where an siRNA shows at least 2 homology regions in the 3'UTR of a gene. The output tables for the test and control set can be found in Figure 11 and 12.

Gene...	...	SubjectN...	SubjectDesc	D	...	Subj...	CDS
ZNF673	10608	NM_006454	Homo sapiens MAX dimerization protein 4 (MXD4), mRNA.	37	8-18	2839-2849	33-662
ZNF673	10608	NM_006454	Homo sapiens MAX dimerization protein 4 (MXD4), mRNA.	36	1-18	1681-1698	33-662
LOC147942	9092	NM_005146	Homo sapiens squamous cell carcinoma antigen recognized by T cells(SART1), mRNA.	37	8-18	2979-2989	93-2495
LOC147942	9092	NM_005146	Homo sapiens squamous cell carcinoma antigen recognized by T cells(SART1), mRNA.	33	7-18	2876-2887	93-2495
GNA11	9681	NM_001007188	Homo sapiens DEP domain containing 5 (DEPDC5), transcript variant 2,mRNA.	41	9-18	1898-1907	143-1822
GNA11	9681	NM_001007188	Homo sapiens DEP domain containing 5 (DEPDC5), transcript variant 2,mRNA.	41	9-18	2047-2056	143-1822
C10orf95	116519	NM_052968	Homo sapiens apolipoprotein A-V (APOA5), transcript variant 1, mRNA.	39	4-18	1506-1520	74-1174
C10orf95	116519	NM_052968	Homo sapiens apolipoprotein A-V (APOA5), transcript variant 1, mRNA.	39	4-18	1506-1520	74-1174
C10orf95	116519	NM_001166598	Homo sapiens apolipoprotein A-V (APOA5), transcript variant 2, mRNA.	39	4-18	1482-1496	50-1150
C10orf95	116519	NM_001166598	Homo sapiens apolipoprotein A-V (APOA5), transcript variant 2, mRNA.	39	4-18	1482-1496	50-1150
TPD52L2	9790	NM_014753	Homo sapiens BMS1 homolog, ribosome assembly protein (yeast) (BMS1),mRNA.	51	8-19	5573-5584	359-4207
TPD52L2	9790	NM_014753	Homo sapiens BMS1 homolog, ribosome assembly protein (yeast) (BMS1),mRNA.	41	10-19	5325-5334	359-4207
TPD52L2	6059	NM_002940	Homo sapiens ATP-binding cassette, sub-family E (OABP), member 1(ABCE1), transcript variant 1, ...	38	6-18	2287-2299	444-2243
TPD52L2	6059	NM_002940	Homo sapiens ATP-binding cassette, sub-family E (OABP), member 1(ABCE1), transcript variant 1, ...	37	8-18	3299-3309	444-2243
TPD52L2	6059	NM_001040876	Homo sapiens ATP-binding cassette, sub-family E (OABP), member 1(ABCE1), transcript variant 2, ...	38	6-18	2284-2296	441-2240
TPD52L2	6059	NM_001040876	Homo sapiens ATP-binding cassette, sub-family E (OABP), member 1(ABCE1), transcript variant 2, ...	37	8-18	3296-3306	441-2240
DTNBP1	6176	NM_213725	Homo sapiens ribosomal protein, large, P1 (RPLP1), transcriptvariant 2, mRNA.	55	8-18	425-435	130-399
DTNBP1	6176	NM_213725	Homo sapiens ribosomal protein, large, P1 (RPLP1), transcriptvariant 2, mRNA.	55	8-18	425-435	130-399
DTNBP1	6176	NM_001003	Homo sapiens ribosomal protein, large, P1 (RPLP1), transcriptvariant 1, mRNA.	55	8-18	500-510	130-474
DTNBP1	6176	NM_001003	Homo sapiens ribosomal protein, large, P1 (RPLP1), transcriptvariant 1, mRNA.	55	8-18	500-510	130-474

Fig.11. Test set of the first dataset applied to the off-target analysis, searching for homologies against a top hit of the screen.

Gen...	siR...	...	SubjectN...	SubjectDesc	D	...	Subje...	CDS
NR6A1	CTGGAA...	4928	NM_139132	Homo sapiens nucleoporin 98kDa (NUP98), transcript variant 4, mRNA.	43	5-18	6587-6600	292-5472
NR6A1	CTGGAA...	4928	NM_139132	Homo sapiens nucleoporin 98kDa (NUP98), transcript variant 4, mRNA.	43	5-18	6587-6600	292-5472
NR6A1	CTGGAA...	4928	NM_016320	Homo sapiens nucleoporin 98kDa (NUP98), transcript variant 1, mRNA.	43	5-18	6809-6822	292-5694
NR6A1	CTGGAA...	4928	NM_016320	Homo sapiens nucleoporin 98kDa (NUP98), transcript variant 1, mRNA.	43	5-18	6809-6822	292-5694
NR6A1	CTGGAA...	9730	NM_014703	Homo sapiens Vpr (HIV-1) binding protein (VPRBP), transcript variant1, mRNA.	47	7-19	5975-5987	186-4709
NR6A1	CTGGAA...	9730	NM_014703	Homo sapiens Vpr (HIV-1) binding protein (VPRBP), transcript variant1, mRNA.	47	7-19	5975-5987	186-4709
NR6A1	CTGGAA...	6059	NM_002940	Homo sapiens ATP-binding cassette, sub-family E (OABP), member 1(ABCE1), transcript variant 1, ...	50	9-18	3807-3816	444-2243
NR6A1	CTGGAA...	6059	NM_002940	Homo sapiens ATP-binding cassette, sub-family E (OABP), member 1(ABCE1), transcript variant 1, ...	47	6-18	3593-3605	444-2243
NR6A1	CTGGAA...	5683	NM_002787	Homo sapiens proteasome (prosome, macropain) subunit, alpha type, 2(PSMA2), mRNA.	42	8-19	1311-1322	49-753
NR6A1	CTGGAA...	5683	NM_002787	Homo sapiens proteasome (prosome, macropain) subunit, alpha type, 2(PSMA2), mRNA.	42	8-19	1311-1322	49-753
NR6A1	CTGGAA...	91584	NM_001105543	Homo sapiens plexin A4 (PLXNA4), transcript variant 3, mRNA.	41	9-18	3367-3376	230-1708
NR6A1	CTGGAA...	91584	NM_001105543	Homo sapiens plexin A4 (PLXNA4), transcript variant 3, mRNA.	41	9-18	3367-3376	230-1708
NR6A1	CTGGAA...	6059	NM_001040876	Homo sapiens ATP-binding cassette, sub-family E (OABP), member 1(ABCE1), transcript variant 2, ...	50	9-18	3804-3813	441-2240
NR6A1	CTGGAA...	6059	NM_001040876	Homo sapiens ATP-binding cassette, sub-family E (OABP), member 1(ABCE1), transcript variant 2, ...	47	6-18	3590-3602	441-2240
NR6A1	CTGGAA...	6165	NM_000996	Homo sapiens ribosomal protein L35a (RPL35A), mRNA.	38	6-18	414-426	74-406
NR6A1	CTGGAA...	6165	NM_000996	Homo sapiens ribosomal protein L35a (RPL35A), mRNA.	38	6-18	414-426	74-406
NR6A1	CTGGAA...	5740	NM_000961	Homo sapiens prostaglandin I2 (prostacyclin) synthase (PTGIS), mRNA.	42	8-19	5471-5482	55-1557
NR6A1	CTGGAA...	5740	NM_000961	Homo sapiens prostaglandin I2 (prostacyclin) synthase (PTGIS), mRNA.	37	8-18	5522-5532	55-1557
LOC728297	CAGAAT...	10050	NM_005495	Homo sapiens solute carrier family 17 (sodium phosphate), member 4(SLC17A4), mRNA.	42	8-19	3242-3253	120-1613
LOC728297	CAGAAT...	10050	NM_005495	Homo sapiens solute carrier family 17 (sodium phosphate), member 4(SLC17A4), mRNA.	41	10-19	3543-3552	120-1613
LOC728297	AGGCTC...	51585	NM_015885	Homo sapiens PCF11, cleavage and polyadenylation factor subunit,homolog (S. cerevisiae) (PCF11)...	46	8-18	5149-5159	346-5013
LOC728297	AGGCTC...	51585	NM_015885	Homo sapiens PCF11, cleavage and polyadenylation factor subunit,homolog (S. cerevisiae) (PCF11)...	45	10-18	5297-5305	346-5013
LOC728297	AGGCTC...	51585	NM_015885	Homo sapiens PCF11, cleavage and polyadenylation factor subunit,homolog (S. cerevisiae) (PCF11)...	38	6-18	5887-5899	346-5013
LOC728297	AGGCTC...	5683	NM_002787	Homo sapiens proteasome (prosome, macropain) subunit, alpha type, 2(PSMA2), mRNA.	38	6-18	1303-1315	49-753
LOC728297	AGGCTC...	5683	NM_002787	Homo sapiens proteasome (prosome, macropain) subunit, alpha type, 2(PSMA2), mRNA.	38	6-18	1303-1315	49-753
LOC728297	AGGCTC...	5740	NM_000961	Homo sapiens prostaglandin I2 (prostacyclin) synthase (PTGIS), mRNA.	49	2-18	3077-3093	55-1557
LOC728297	AGGCTC...	5740	NM_000961	Homo sapiens prostaglandin I2 (prostacyclin) synthase (PTGIS), mRNA.	49	2-18	3077-3093	55-1557

Fig.12. Control set of the first dataset applied to the off-target analysis, searching for homologies against a top hit of the screen.

The intention to show that the siRNAs of the test set have more significant matches with top hits than the siRNAs of the control set was not successful. The output table for the test set contains 20 entries, with potential off-target gene for 6 out of the 13 oligos. The result list of the control set contains 27 entries and for 3 out of the 12 oligos potential candidates are found. Hence, a significant difference in size between these two lists does not exist. This observation is also confirmed by a further analysis workflow performed with the test and the control set of the second dataset. This workflow with its configuration settings is shown in Figure 13, as well as the corresponding output tables of both sets (see on figure 14 for the test set and figure 15 for the control set).

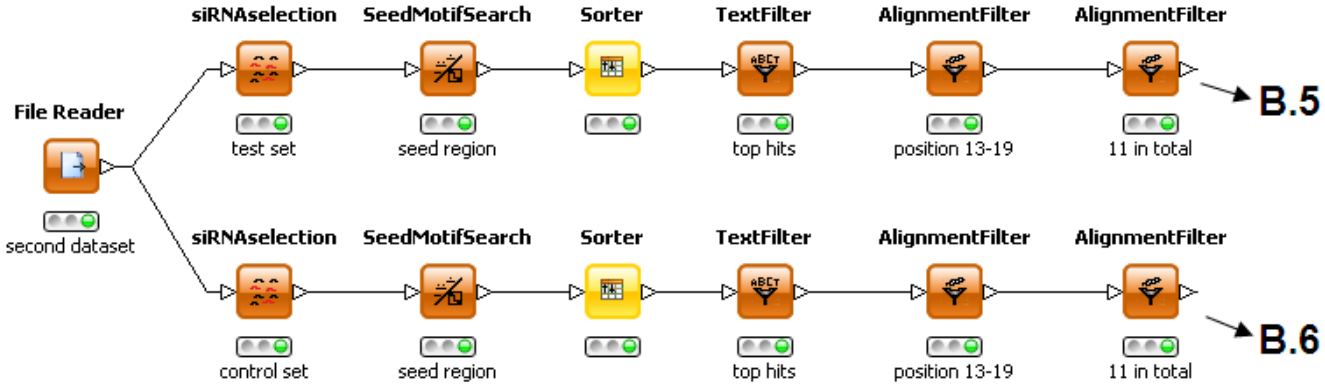


Fig.13. Workflow to analyse off-target effects with the second test and control set.

NCBI...	...	Subject...	SubjectDesc	D ...	S ...	Subje...	CDS
TBL3	391	NM_001665	Homo sapiens ras homolog gene family, member G (rho G) (RHOG), mRNA.	59	1-19	425-443	159-734
TBL3	9978	NM_014248	Homo sapiens ring-box 1 (RBX1), mRNA.	43	6-19	43-56	19-345
TBL3	10810	NM_006646	Homo sapiens WAS protein family, member 3 (WASF3), mRNA.	41	1-19	2590-2608	226-1734
TSPO	22820	NM_016128	Homo sapiens coatomer protein complex, subunit gamma (COPG), mRNA.	52	6-19	1762-1775	101-2725
TSPO	476	NM_000701	Homo sapiens ATPase, Na+/K+ transporting, alpha 1 polypeptide(ATP1A1), transcript variant 1, mRNA.	40	3-19	75-91	340-3411
PSMB8	5696	NM_004159	Homo sapiens proteasome (prosome, macropain) subunit, beta type, 8(large multifunctional peptidase 7) (PSMB8), transcript variant 1...	95	1-19	1156-1174	523-1341
PSMB8	5696	NM_148919	Homo sapiens proteasome (prosome, macropain) subunit, beta type, 8(large multifunctional peptidase 7) (PSMB8), transcript variant 2...	95	1-19	689-707	44-874
PSMB8	5781	NM_002834	Homo sapiens protein tyrosine phosphatase, non-receptor type 11(PTPN11), mRNA.	45	2-19	4084-4101	381-2162
PSMB8	526	NM_001693	Homo sapiens ATPase, H+ transporting, lysosomal 56/58kDa, V1 subunitB2 (ATP6V1B2), mRNA.	44	4-19	2793-2808	215-1750
ARFGEF1	10097	NM_001005386	Homo sapiens ARP2 actin-related protein 2 homolog (yeast) (ACTR2),transcript variant 1, mRNA.	52	6-19	3676-3689	216-1415
ARFGEF1	10097	NM_005722	Homo sapiens ARP2 actin-related protein 2 homolog (yeast) (ACTR2),transcript variant 2, mRNA.	52	6-19	3661-3674	216-1400
ARFGEF1	8976	NM_003941	Homo sapiens Wiskott-Aldrich syndrome-like (WASL), mRNA.	51	8-19	3264-3275	329-1846
ARFGEF1	10096	NM_005721	Homo sapiens ARP3 actin-related protein 3 homolog (yeast) (ACTR3),mRNA.	49	3-19	1224-1240	321-1577
ARFGEF1	7879	NM_004637	Homo sapiens RAB7A, member RAS oncogene family (RAB7A), mRNA.	47	7-19	1611-1623	233-856

Fig.14. Test set of the second dataset applied to the off-target analysis, searching for homologies against a top hit of the screen.

NC...	siRN...	S...	Subject...	SubjectDesc	D ...	S ...	Subj...	CDS
GIT1	CCGGATC...	5216	NM_005022	Homo sapiens profilin 1 (PFN1), mRNA.	43	6-19	313-326	137-559
NUCKS1	AGGAAG...	5781	NM_002834	Homo sapiens protein tyrosine phosphatase, non-receptor type 11(PTPN11), mRNA.	49	3-19	551-567	381-2162
NUCKS1	AGGAAG...	10096	NM_005721	Homo sapiens ARP3 actin-related protein 3 homolog (yeast) (ACTR3),mRNA.	43	6-19	2200-2213	321-1577
NUCKS1	AGGAAG...	10810	NM_006646	Homo sapiens WAS protein family, member 3 (WASF3), mRNA.	40	3-19	954-970	226-1734
NUCKS1	AGGAAG...	5683	NM_002787	Homo sapiens proteasome (prosome, macropain) subunit, alpha type, 2(PSMA2), mRNA.	39	5-19	722-736	49-753
NUCKS1	AAGAAC...	122706	NM_001099...	Homo sapiens proteasome (prosome, macropain) subunit, beta type, 11(PSMB11), mRNA.	39	5-19	875-889	60-962

Fig.15. Control set of the second dataset applied to the off-target analysis, searching for homologies against a top hit of the screen.

Further tests with a variety of filter combinations amplify the impression that a comparison between two siRNA sets is not helpful to determine the reliability of the results. So although some off-target candidates after the use of the Text Filter are meaningful and would explain the occurrence of the effect in the screen, further validation strategies have to be performed to determine the effectiveness of the software components and the reliability the results.

I. Detection of the divergent oligo.

One possibility to validate the new toolkit is to take a gene which belongs to the 1/4 category in the screening results, i.e. only one oligo out of the four shows an effect. Such a gene in the first dataset is SOX17. The idea is to detect the oligo which shows the observed effect by performing the off-target analysis. The following workflow is performed to find the divergent oligo of SOX17. Initially, the four oligos are applied to the default Seed-Motif-Search Node for the homology search. Afterwards two Text Filter nodes work in parallel and filter the output table for the top (4/4) and the 3/4 hits. The result lists are concatenated and further reduced with two Alignment Filter nodes so that only those results are included which contain matches near the seed region (bases 13-19) and matches in total. Thus it is ensured that the alignments are quite compact and not too short. The output list after this filtering is shown in figure 16.

G...	Genename	siRNA Sequence	Su...	SubjectN...	SubjectDesc	D	S ...	Subje...
SOX17	SRY (sex determining region Y)-box 17	CTCCATTTCCTGAAAGTTTAT	124739	NM_153210	Homo sapiens ubiquitin specific peptidase 43 (USP43), mRNA.	52	6-19	2774-2787
SOX17	SRY (sex determining region Y)-box 17	CTCCATTTCCTGAAAGTTTAT	83443	NM_031287	Homo sapiens splicing factor 3b, subunit 5, 10kDa (SF3B5), mRNA.	52	6-19	709-722
SOX17	SRY (sex determining region Y)-box 17	CTCCATTTCCTGAAAGTTTAT	57050	NM_020368	Homo sapiens UTP3, small subunit (SSU) processome component, homolog(S. cerevisiae) (UTP3), ...	39	5-19	816-830
SOX17	SRY (sex determining region Y)-box 17	CTCCATTTCCTGAAAGTTTAT	124739	NM_153210	Homo sapiens ubiquitin specific peptidase 43 (USP43), mRNA.	39	5-19	3950-3964
SOX17	SRY (sex determining region Y)-box 17	CTCCATTTCCTGAAAGTTTAT	3550	NM_006083	Homo sapiens IK cytokine, down-regulator of HLA II (IK), mRNA.	36	2-19	1335-1352
SOX17	SRY (sex determining region Y)-box 17	CCGCACGGAATTTGAACAGTA	6059	NM_002940	Homo sapiens ATP-binding cassette, sub-family E (OABP), member 1(ABCE1), transcript variant 1...	47	7-19	3304-3316
SOX17	SRY (sex determining region Y)-box 17	CCGCACGGAATTTGAACAGTA	6059	NM_001040876	Homo sapiens ATP-binding cassette, sub-family E (OABP), member 1(ABCE1), transcript variant 2...	47	7-19	3301-3313
SOX17	SRY (sex determining region Y)-box 17	AAACCCTATTTCCAAGTTCAA	116519	NM_001166598	Homo sapiens apolipoprotein A-V (APOA5), transcript variant 2, mRNA.	45	2-19	242-259
SOX17	SRY (sex determining region Y)-box 17	AAACCCTATTTCCAAGTTCAA	116519	NM_052968	Homo sapiens apolipoprotein A-V (APOA5), transcript variant 1, mRNA.	45	2-19	266-283
SOX17	SRY (sex determining region Y)-box 17	AAACCCTATTTCCAAGTTCAA	1662	NM_004398	Homo sapiens DEAD (Asp-Glu-Ala-Asp) box polypeptide 10 (DDX10),mRNA.	39	5-19	2993-3007

Fig.16. The four oligos of SOX17 are applied to the Seed-Motif-Search Node (default parameters), two Text Filter nodes (4/4 and 3/4 hits) and two Alignment Filter nodes (matches at position 13-19 and 11 in total).

The first oligo of the result table starting with the bases CT seems to have the most probable off-target gene. It shows the best homology to a top hit in contrast to the other three siRNAs, the alignment score is quite high and also the alignment itself is long and compact ranging from bases 6-19. The hit rates of the four oligos of SOX17 in the first dataset are the following:

Oligo 1: 0.02619 CCGCACGGAATTTGAACAGTA Oligo 2: 0.0039841 TTGGCATATAATTTATGGTAAOligo 3: 0.85238 CTCCATTTCCTGAAAGTTTATOligo 4: 0.042222 AAACCCTATTTCCAAGTTCAAThe workflow analysis of these four oligos of SOX17 revealed that the oligo starting with the bases CT can show an off-target effect in the screen due to a great homology with a top hit. This hypothesis coincides with the content of the

above hit rate table, because the third oligo, CTCCATTTCCTGAAAGTTTAT, has the highest hit rate with 0.85. Hence, with this gene it was possible to detect the divergent oligo out of the four. To prove that the result of the SOX17 analysis did not arise by chance, a great number of further experiments has to be performed in the same way as described above. Such a series of experiments should validate the analyses performed with this new toolkit leading to a statement of confidence about the reliability of the results, e.g. in how many cases the oligo with the high hit rate is detected. As a first step this was already done with 10 genes belonging to the 1/4 category. Thereby, it was possible to detect the divergent oligo in 7 out of the 10 cases (STX11,NEB,EP300,KIF22,SPIC,SAA2,PUM2/APOE,GNG3,CANX).

The analysis of off-target effects in RNAi screening data can be performed by applying different filtering combinations. Depending on constraints and the number of nodes used for the filtering, the off-target genes for a particular oligo might vary. Especially the use of the Text Filter to find homology regions between an siRNA and a top hit turns out to be quite helpful in the analytic process. Unfortunately, the question of reliability of these results remains open and also a comparison between a test and a control does not give any additional insight. Therefore, further investigations are necessary to validate the application of the developed toolkit and its results.

VI. Effect in two out of four oligos

In RNAi screens quite often only two out of the four oligos of a gene show an effect. This second subsection is dealing with this type of genes trying to detect the causes of the observed phenomena. In the following two genes of the provided dataset are selected which fall in this 2/4 hit category. All four oligos are further examined in the workflow environment. The first gene is called FDPSL2A and the hit rates of the four corresponding oligos are shown in the following table:

Oligo 1: 0.79734 AAGGCACAAATCTGTTGTCAA
Oligo 2: 0.025281 CACGTTATGGGTCTCATCGAA
Oligo 3: 0.69014 CAGCTTCTCCAGAACCAAGAA
Oligo 4: 0.025341 ACGGGCCTACCCAGAGGGCAA

These four oligos are analysed for homology regions with mRNAs and the resulting list is further investigated by applying a couple of filter nodes. One example of these filter combinations is the reduction of the initial list to the miRNA-like off-target effects occurring with a top hit gene. Therefore, first two Text Filter nodes to filter for top hits are used followed by the Alignment Filter to ensure that the seed region matches completely. Subsequently, the Location Filter is applied to get the alignments located in the 3'UTR of the mRNA in combination with the Multiple Hit Filter. This filter selects only the siRNA results in which at least two homology regions occur in one mRNA. The result list of this workflow is shown in 17

Ge...	siRNA Sequence	...	SubjectN...	SubjectDesc	D	...	Subject...
FDPSL2A	CAGCTTCTCCAGAACCAAGAA	23521	NM_012423	Homo sapiens ribosomal protein L13a (RPL13A), mRNA.	42	8-19	1048-1059
FDPSL2A	CAGCTTCTCCAGAACCAAGAA	23521	NM_012423	Homo sapiens ribosomal protein L13a (RPL13A), mRNA.	42	8-19	1048-1059
FDPSL2A	CACGTTATGGGTCTCATCGAA	59286	NM_024292	Homo sapiens ubiquitin-like 5 (UBL5), transcript variant 1, mRNA.	30	13-18	416-421
FDPSL2A	CACGTTATGGGTCTCATCGAA	59286	NM_024292	Homo sapiens ubiquitin-like 5 (UBL5), transcript variant 1, mRNA.	30	13-18	448-453
FDPSL2A	CACGTTATGGGTCTCATCGAA	91584	NM_020911	Homo sapiens plexin A4 (PLXNA4), transcript variant 1, mRNA.	37	8-18	7113-7123
FDPSL2A	CACGTTATGGGTCTCATCGAA	91584	NM_020911	Homo sapiens plexin A4 (PLXNA4), transcript variant 1, mRNA.	37	8-18	7113-7123
FDPSL2A	CACGTTATGGGTCTCATCGAA	91584	NM_020911	Homo sapiens plexin A4 (PLXNA4), transcript variant 1, mRNA.	30	13-18	7869-7874
FDPSL2A	CACGTTATGGGTCTCATCGAA	91584	NM_020911	Homo sapiens plexin A4 (PLXNA4), transcript variant 1, mRNA.	30	13-18	12067-12072
FDPSL2A	CACGTTATGGGTCTCATCGAA	59286	NM_001048241	Homo sapiens ubiquitin-like 5 (UBL5), transcript variant 2, mRNA.	30	13-18	336-341
FDPSL2A	CACGTTATGGGTCTCATCGAA	59286	NM_001048241	Homo sapiens ubiquitin-like 5 (UBL5), transcript variant 2, mRNA.	30	13-18	368-373
FDPSL2A	CACGTTATGGGTCTCATCGAA	5740	NM_000961	Homo sapiens prostaglandin I2 (prostacyclin) synthase (PTGIS), mRNA.	38	6-18	5314-5326
FDPSL2A	CACGTTATGGGTCTCATCGAA	5740	NM_000961	Homo sapiens prostaglandin I2 (prostacyclin) synthase (PTGIS), mRNA.	36	11-19	3799-3807
FDPSL2A	ACGGGCCTACCCAGAGGGCAA	91584	NM_020911	Homo sapiens plexin A4 (PLXNA4), transcript variant 1, mRNA.	40	11-18	6591-6598
FDPSL2A	ACGGGCCTACCCAGAGGGCAA	91584	NM_020911	Homo sapiens plexin A4 (PLXNA4), transcript variant 1, mRNA.	35	13-19	7946-7952
FDPSL2A	ACGGGCCTACCCAGAGGGCAA	91584	NM_020911	Homo sapiens plexin A4 (PLXNA4), transcript variant 1, mRNA.	33	7-18	12786-12797
FDPSL2A	ACGGGCCTACCCAGAGGGCAA	91584	NM_020911	Homo sapiens plexin A4 (PLXNA4), transcript variant 1, mRNA.	30	13-18	11394-11399
FDPSL2A	ACGGGCCTACCCAGAGGGCAA	9730	NM_014703	Homo sapiens Vpr (HIV-1) binding protein (VPRBP), transcript variant1, mRNA.	30	13-18	4749-4754
FDPSL2A	ACGGGCCTACCCAGAGGGCAA	9730	NM_014703	Homo sapiens Vpr (HIV-1) binding protein (VPRBP), transcript variant1, mRNA.	30	13-18	5590-5595
FDPSL2A	ACGGGCCTACCCAGAGGGCAA	23450	NM_012426	Homo sapiens splicing factor 3b, subunit 3, 130kDa (SF3B3), mRNA.	45	11-19	4834-4842
FDPSL2A	ACGGGCCTACCCAGAGGGCAA	23450	NM_012426	Homo sapiens splicing factor 3b, subunit 3, 130kDa (SF3B3), mRNA.	45	11-19	7744-7752
FDPSL2A	ACGGGCCTACCCAGAGGGCAA	23450	NM_012426	Homo sapiens splicing factor 3b, subunit 3, 130kDa (SF3B3), mRNA.	36	11-19	6597-6605
FDPSL2A	ACGGGCCTACCCAGAGGGCAA	23450	NM_012426	Homo sapiens splicing factor 3b, subunit 3, 130kDa (SF3B3), mRNA.	35	12-18	5132-5138
FDPSL2A	ACGGGCCTACCCAGAGGGCAA	23450	NM_012426	Homo sapiens splicing factor 3b, subunit 3, 130kDa (SF3B3), mRNA.	35	12-18	5823-5829
FDPSL2A	ACGGGCCTACCCAGAGGGCAA	10608	NM_006454	Homo sapiens MAX dimerization protein 4 (MXD4), mRNA.	31	11-18	3679-3686
FDPSL2A	ACGGGCCTACCCAGAGGGCAA	10608	NM_006454	Homo sapiens MAX dimerization protein 4 (MXD4), mRNA.	30	13-18	1518-1523
FDPSL2A	ACGGGCCTACCCAGAGGGCAA	5740	NM_000961	Homo sapiens prostaglandin I2 (prostacyclin) synthase (PTGIS), mRNA.	30	13-18	1721-1726
FDPSL2A	ACGGGCCTACCCAGAGGGCAA	5740	NM_000961	Homo sapiens prostaglandin I2 (prostacyclin) synthase (PTGIS), mRNA.	30	13-18	4165-4170
FDPSL2A	AAGGCACAAATCTGTTGTCAA	51077	NM_015962	Homo sapiens FCF1 small subunit (SSU) processome component homolog(S. cerevisiae) (FCF1)...	40	11-18	2207-2214
FDPSL2A	AAGGCACAAATCTGTTGTCAA	51077	NM_015962	Homo sapiens FCF1 small subunit (SSU) processome component homolog(S. cerevisiae) (FCF1)...	31	11-18	1608-1615
FDPSL2A	AAGGCACAAATCTGTTGTCAA	23521	NM_012423	Homo sapiens ribosomal protein L13a (RPL13A), mRNA.	35	12-18	1002-1008
FDPSL2A	AAGGCACAAATCTGTTGTCAA	23521	NM_012423	Homo sapiens ribosomal protein L13a (RPL13A), mRNA.	35	12-18	1002-1008
FDPSL2A	AAGGCACAAATCTGTTGTCAA	91584	NM_001105543	Homo sapiens plexin A4 (PLXNA4), transcript variant 3, mRNA.	37	9-19	1739-1749
FDPSL2A	AAGGCACAAATCTGTTGTCAA	91584	NM_001105543	Homo sapiens plexin A4 (PLXNA4), transcript variant 3, mRNA.	35	13-19	2366-2372

Fig.17. The four oligos of FDPSL2A are applied to the Seed-Motif-Search Node (default parameters), two Text Filter nodes (4/4 and 3/4 hits), the Alignment Filter (matches at position 13-18, the Location Filter (3'UTR of the mRNA) and the Multiple Hit Filter (at least 2 times).

The results of the described analysis workflow reveal no significant difference between the four oligos of FDPSL2A. Oligo 1 and 3, those with the high hit rates, do not show any specific properties in comparison to oligo 2 and 4. All four oligos have multiple homology regions with an mRNA and also the alignment scores are pretty much the same. Hence, those oligos with a high hit rate probably work accurately and silence the target gene (FDPSL2A). In contrast, the other two oligos might fail to knockdown the target gene, perhaps due to experimental conditions. Further analyses are necessary to investigate this hypothesis.

A second example is the gene ovostatin 2, OVOS2, with the following four oligos:

Oligo 1: 0.061404 TAGGCCTGATATTAATAGCAA

Oligo 2: 0.60606 ATCCAGTGAGATCCAGTTTAA

Oligo 3: 0.97727 CTGCTGATTCCTTCTGTTCTA

Oligo 4: 0 TCCAACCAGGTGATTAATGTA

These four oligos of OVOS2 are applied to the same off-target analysis workflow used in the example before to explore the siRNAs of FDPSL2A. At the beginning a homology search is performed with the default Seed-Motif-Search Node, followed by two Text Filter nodes to obtain homologies with a top hit. As a next filtering step the Alignment Filer is used to get the alignments containing matches at the seed region. Afterwards the Location Filter (3'UTR) in combination with the Multiple Hit Filter (value = 2) is applied. The result table of this miRNAlike functional analysis of the four OVOS2 oligos is shown in figure_18

.

G...	siRNA Sequence	...	SubjectN...	SubjectDesc	D	...	Subject...
OVOS2	CTGCTGATTCCTTCTGTTCTA	91584	NM_020911	Homo sapiens plexin A4 (PLXNA4), transcript variant 1, mRNA.	50	10-19	12132-12141
OVOS2	CTGCTGATTCCTTCTGTTCTA	91584	NM_020911	Homo sapiens plexin A4 (PLXNA4), transcript variant 1, mRNA.	41	10-19	7323-7332
OVOS2	CTGCTGATTCCTTCTGTTCTA	91584	NM_020911	Homo sapiens plexin A4 (PLXNA4), transcript variant 1, mRNA.	40	3-19	12626-12642
OVOS2	CTGCTGATTCCTTCTGTTCTA	91584	NM_020911	Homo sapiens plexin A4 (PLXNA4), transcript variant 1, mRNA.	40	11-18	12670-12677
OVOS2	CTGCTGATTCCTTCTGTTCTA	91584	NM_020911	Homo sapiens plexin A4 (PLXNA4), transcript variant 1, mRNA.	36	10-18	11556-11564
OVOS2	CTGCTGATTCCTTCTGTTCTA	91584	NM_020911	Homo sapiens plexin A4 (PLXNA4), transcript variant 1, mRNA.	30	13-18	7506-7511
OVOS2	CTGCTGATTCCTTCTGTTCTA	55234	NM_018225	Homo sapiens smu-1 suppressor of mec-8 and unc-52 homolog (C.elegans) (SMU1)...	40	12-19	2024-2031
OVOS2	CTGCTGATTCCTTCTGTTCTA	55234	NM_018225	Homo sapiens smu-1 suppressor of mec-8 and unc-52 homolog (C.elegans) (SMU1)...	40	11-18	3862-3869
OVOS2	CTGCTGATTCCTTCTGTTCTA	55234	NM_018225	Homo sapiens smu-1 suppressor of mec-8 and unc-52 homolog (C.elegans) (SMU1)...	40	12-19	6173-6180
OVOS2	CTGCTGATTCCTTCTGTTCTA	55234	NM_018225	Homo sapiens smu-1 suppressor of mec-8 and unc-52 homolog (C.elegans) (SMU1)...	30	13-18	2877-2882
OVOS2	CTGCTGATTCCTTCTGTTCTA	55234	NM_018225	Homo sapiens smu-1 suppressor of mec-8 and unc-52 homolog (C.elegans) (SMU1)...	30	13-18	3114-3119
OVOS2	CTGCTGATTCCTTCTGTTCTA	55234	NM_018225	Homo sapiens smu-1 suppressor of mec-8 and unc-52 homolog (C.elegans) (SMU1)...	30	13-18	6569-6574
OVOS2	CTGCTGATTCCTTCTGTTCTA	23450	NM_012426	Homo sapiens splicing factor 3b, subunit 3, 130kDa (SF3B3), mRNA.	35	12-18	6221-6227
OVOS2	CTGCTGATTCCTTCTGTTCTA	23450	NM_012426	Homo sapiens splicing factor 3b, subunit 3, 130kDa (SF3B3), mRNA.	35	12-18	6851-6857
OVOS2	CTGCTGATTCCTTCTGTTCTA	23450	NM_012426	Homo sapiens splicing factor 3b, subunit 3, 130kDa (SF3B3), mRNA.	35	13-19	7662-7668
OVOS2	CTGCTGATTCCTTCTGTTCTA	23450	NM_012426	Homo sapiens splicing factor 3b, subunit 3, 130kDa (SF3B3), mRNA.	30	13-18	5579-5584
OVOS2	CTGCTGATTCCTTCTGTTCTA	23450	NM_012426	Homo sapiens splicing factor 3b, subunit 3, 130kDa (SF3B3), mRNA.	30	13-18	7284-7289
OVOS2	CTGCTGATTCCTTCTGTTCTA	9939	NM_005105	Homo sapiens RNA binding motif protein 8A (RBM8A), mRNA.	40	12-19	1177-1184
OVOS2	CTGCTGATTCCTTCTGTTCTA	9939	NM_005105	Homo sapiens RNA binding motif protein 8A (RBM8A), mRNA.	40	12-19	1177-1184
OVOS2	CTGCTGATTCCTTCTGTTCTA	9939	NM_005105	Homo sapiens RNA binding motif protein 8A (RBM8A), mRNA.	31	11-18	560-567
OVOS2	CTGCTGATTCCTTCTGTTCTA	9939	NM_005105	Homo sapiens RNA binding motif protein 8A (RBM8A), mRNA.	30	13-18	2623-2628
OVOS2	CTGCTGATTCCTTCTGTTCTA	6229	NM_001142285	Homo sapiens ribosomal protein S24 (RPS24), transcript variant d,mRNA.	36	11-19	1126-1134
OVOS2	CTGCTGATTCCTTCTGTTCTA	6229	NM_001142285	Homo sapiens ribosomal protein S24 (RPS24), transcript variant d,mRNA.	34	5-18	2132-2145
OVOS2	CTGCTGATTCCTTCTGTTCTA	6229	NM_001142285	Homo sapiens ribosomal protein S24 (RPS24), transcript variant d,mRNA.	30	13-18	1788-1793
OVOS2	ATCCAGTGAGATCCAGTTTAA	23450	NM_012426	Homo sapiens splicing factor 3b, subunit 3, 130kDa (SF3B3), mRNA.	45	11-19	9628-9636
OVOS2	ATCCAGTGAGATCCAGTTTAA	23450	NM_012426	Homo sapiens splicing factor 3b, subunit 3, 130kDa (SF3B3), mRNA.	37	8-18	4876-4886
OVOS2	ATCCAGTGAGATCCAGTTTAA	23450	NM_012426	Homo sapiens splicing factor 3b, subunit 3, 130kDa (SF3B3), mRNA.	35	13-19	7759-7765

Fig.18. The four oligos of OVOS2 are applied to the Seed-Motif-Search Node (default parameters), two Text Filter nodes (4/4 and 3/4 hits), the Alignment Filters (matches at position 13-18, the Location Filter (3'UTR of the mRNA) and the Multiple Hit Filter (at least 2 times).

This output table clearly shows that two out of the four oligos have potential off-target genes which can be silenced in a miRNA-like manner. These oligos, starting with the bases CT and AT, are also those siRNAs which have a high hit rate in the screen. The other two oligos with a low hit rate are not included in the result list of the off-target analysis. So in this example the divergent effects of the OVOS2 oligos occurring in the screening results may be ascribed to miRNA-like off-target effects.

Taking all four oligos of the 2/4 candidates and applying the same analysis workflow turned out to be a useful variant for their examination. The comparison of the off-target results between the four siRNAs leads to hypotheses why these divergent effects occur in the screen. However, the reliability of the results is still an open question as in the previous subsection. Therefore further analyses and experiments are recommended and necessary to verify the hypotheses.

VII. Conclusion

The major objective of this paper was to describe a kernel method to analyse off-target effects in RNAi screening data. The creation of flexible workflows by combining for example different filtering nodes is a big advantage and allows the performance of specific analyses. However, the runtime problem caused by the Smith-Waterman algorithm had a great influence on the development of the underlying concept. To circumvent this problem two limitations were

necessary in the homology search, either using only a subset of mRNAs or adding a seed-motif search step before. Both limitations are nevertheless meaningful and do not have a great impact on the final result. The subset of mRNA could contain for example only top hit genes of the screen and a homology with these genes would explain an effect. The restriction of the seed region also seems to be a good starting point. In the final implementation this restriction is lessened, because start and end positions of the motif are not fixed to the seed region and thus can be variably configured. The general mapping of individual tasks to single nodes which can be executed independently makes the toolkit easily extensible by either adding a new node or by modifying the functionality of an existing one. Since current research is intensively working on revealing more indicators for the occurrence of off-target effects, this extensibility is especially important for future applications. The test and validation phase has proven that with the current state of the software a powerful and flexible toolkit for analysing off-target effects is already given. Beside the implementation of the software, its test and validation with two datasets has been another very important aspect. It should reveal if the workflow environment is suitable for such a data analysis and whether the aim to find off-target effects influencing the screening results can be achieved or not. The analyses of the given datasets show that the environment allows for a dynamic workflow adaption based on intermediate results, e.g. by supplemental Text Filter integration. In addition to the flexible workflow creation facility, the individual configuration options of a single node are also advantageous. For example the analysis of two datasets with complete different definitions of a hit rate is possible. All in all, the software environment with its flexibility turns out to be very suitable to analyse off-target effects in RNAi screening data. An important aspect is the reliability of the results obtained in the analysis process. Without concrete knowledge of the underlying mechanism, it is hard to say which of the results are false positives and which are correct. Therefore, selective strategies to test the reliability have to be performed.

In such a large-scale analysis many genes have to be selected where only one out of the four oligos shows an effect in the screen. Aim of the strategy is to detect this divergent oligo based on the outcome of the performed off-target analysis. Depending on how often the detection was successful, a conclusion can be drawn about the reliability of the analytical results. The implemented software is just the first step in the complex field of analysing off-target effects. Because of its flexible use in the analytic process and its extensibility features, the developed software provides a suitable toolkit to perform analyses of off-target effects in RNAi screening data. In the test and validation process a number of promising results have been found so far which led to the exclusion of some top hits. Before the toolkit can be ultimately integrated into a biologist's day-to-day work, the open question on the reliability of the results has to be answered.

VIII. References

1) Gregory J. Hannon. Rna interference. Nature, 418(6894):244–51, July 2002.
2) Andreas Soejitno, Desak M. Wihandani, and Tuty Kuswardhani. The therapeutic potential sof rna interference in controlling hiv-1 replication. Acta Med Indones, 41(4):215–21, October 2009.
3) Vivek C. Abraham, D. Lansing Taylor, and Jeffrey R. Haskins. High content screening applied to large-scale cell biology. Trends Biotechnol, 22(1):15–22, January 2004.
4) Xiaoyu Lin, Xiaoan Ruan, Mark G. Anderson, Jeffrey A. McDowell, Paul E. Kroeger, Stephen W. Fesik, and Yu Shen. sirna-mediated off-target gene silencing triggered by a 7 nt complementation. Nucleic Acids Res, 33(14):4527–35, 2005.
5) Yuriy Fedorov, Alan King, Emily M. Anderson, Jon Karpilow, Diane Ilsley, William S. Marshall, and Anastasia Khvorova. Different delivery methods-different expression profiles. Nat Methods, 2(4):241, April 2005.
6) Angela Reynolds, Emily M. Anderson, Annaleen Vermeulen, Yuriy Fedorov, Kathryn Robinson, Devin Leake, Jon Karpilow, William S. Marshall, and Anastasia Khvorova. Induction of the interferon response by sirna is cell type- and duplex length-dependent. RNA, 12(6):988–93, June 2006.
7) Carol A. Sledz, Michelle Holko, Michael J. de Veer, Robert H. Silverman, and Bryan R. G. Williams. Activation of the interferon system by short-interfering rnas. Nat Cell Biol, 5(9):834 9, September 2003.
8) Stephan P. Persengiev, Xiaochun Zhu, and Michael R. Green. Nonspecific, concentrationdependent stimulation and repression of mammalian gene expression by small interfering rnas (sirnas). RNA, 10(1):12–8, January 2004.
9) Yuriy Fedorov, Emily M. Anderson, Amanda Birmingham, Angela Reynolds, Jon Karpilow, Kathryn Robinson, Devin Leake, William S. Marshall, and Anastasia Khvorova. Offtarget effects by sirna can induce toxic phenotype. RNA, 12(7):1188–96, July 2006.
10) Aimee L. Jackson, Julja Burchard, Devin Leake, Angela Reynolds, Janell Schelter, Jie Guo, Jason M. Johnson, Lee Lim, Jon Karpilow, Kim Nichols, William S. Marshall, Anastasia Khvorova, and Peter S. Linsley. Position-specific chemical modification of sirnas reduces "off-target" transcript silencing. RNA, 12(7):1197–205, July 2006.
11) Aimee L. Jackson, Steven R. Bartz, Janell Schelter, Sumire V. Kobayashi, Julja Burchard, Mao Mao, Bin Li, Guy Cavet, and Peter S. Linsley. Expression profiling reveals off-target gene regulation by rnai. Nat Biotechnol, 21(6):635–7, June 2003.

12) Jesper B. Bramsen, Maria B. Laursen, Christian K. Damgaard, Suzy W. Lena, B Ravindra Babu, Jesper Wengel, and Jürgen Kjems. Improved silencing properties using small internally segmented interfering rnas. Nucleic Acids Res, 35(17):5886–97, 2007.
13) Dimitri Semizarov, Leigh Frost, Aparna Sarthy, Paul E. Kroeger, Donald N. Halbert, and Stephen W. Fesik. Specificity of short interfering rna determined through gene expression signatures. Proc Natl Acad Sci U S A, 100(11):6347–52, May 2003.
14) Benjamin Haley and Phillip D. Zamore. Kinetic analysis of the rnai enzyme complex. Nat Struct Mol Biol, 11(7):599–606, July 2004.
15) Sayda M. Elbashir, Javier Martinez, Agnieszka Patkaniowska, Winfried Lendeckel, and Thomas Tuschl. Functional anatomy of sirnas for mediating efficient rnai in drosophila melanogaster embryo lysate. EMBO J, 20(23):6877–88, December 2001.
16) [Aimee L. Jackson, Julja Burchard, Janell Schelter, B Nelson Chau, Michele Cleary, Lee Lim, and Peter S. Linsley. Widespread sirna "off-target" transcript silencing mediated by seed region sequence complementarity. RNA, 12(7):1179–87, July 2006.
17) Amanda Birmingham, Emily M. Anderson, Angela Reynolds, Diane Ilsley-Tyree, Devin Leake, Yuriy Fedorov, Scott Baskerville, Elena Maksimova, Kathryn Robinson, Jon Karpilow,
18) William S. Marshall, and Anastasia Khvorova. 3' utr seed matches, but not overall identity, are associated with rnai off-targets. Nat Methods, 3(3):199–204, March 2006.
19) Sandeep Saxena, Zophonías O. Jónsson, and Anindya Dutta. Small rnas with imperfect match to endogenous mrna repress translation. implications for off-target activity of small inhibitory rna in mammalian cells. J Biol Chem, 278(45):44312–9, November 2003.
20) Mohammed Amarzguioui, Torgeir Holen, Eshrat Babaie, and Hans Prydz. Tolerance for mutations and chemical modifications in a sirna. Nucleic Acids Res, 31(2):589–95, January 2003.
21) Benjamin P. Lewis, Christopher B. Burge, and David P. Bartel. Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microrna targets. Cell, 120(1):15–20, January 2005.
22) John G. Doench, Christian P. Petersen, and Phillip A. Sharp. sirnas can function as mirnas. Genes Dev, 17(4):438–42, February 2003.
23) [22] Lourdes M. Alemán, John G. Doench, and Phillip A. Sharp. Comparison of sirna-induced off-target rna and protein effects. RNA, 13(3):385–95, March 2007.
24) Emily M. Anderson, Amanda Birmingham, Scott Baskerville, Angela Reynolds, Elena Maksimova, Devin Leake, Yuriy Fedorov, Jon Karpilow, and Anastasia Khvorova. Experimental validation of the importance of seed complement frequency to sirna specificity. RNA, 14(5):853–61, May 2008.
25) Kumiko Ui-Tei, Yuki Naito, Kenji Nishi, Aya Juni, and Kaoru Saigo. Thermodynamic stability and watson-crick base pairing in the seed duplex are major determinants of the efficiency of the sirna-based off-target effect. Nucleic Acids Res, 36(22):7100–9, December 2008.
26) Stephen F. Altschul, Warren Gish, Webb Miller, Eugene W. Myers, and David J. Lipman. Basic local alignment search tool. J Mol Biol, 215(3):403–10, October 1990.
27) Temple F. Smith and Michael S. Waterman. Identification of common molecular subsequences. J Mol Biol, 147(1):195–7, March 1981.
28) Markus C. Schlumberger and Wolf-Dietrich Hardt. Salmonella type iii secretion effectors: pulling the host cell's strings. Curr Opin Microbiol, 9(1):46–54, February 2006.

The effect of retarding myopia progression with seasonal modification of topical atropine in Chiayi area, Taiwan

GJMR Classification (FOR) 321016,320000,320705

Paul CS Lu[1,2,3] MD .MS. Jackie CL Chen[2] MD

***Abstract*- To determine if different concentration of atropine with seasonal modification is more physiologically effective to retard myopia progression.**

***Methods*— Two hundred and forty eyes of one hundred and twenty healthy preschool- and school-aged children were recruited. The case group (group 1) had one hundred and twenty-six eyes of sixty three children; one hundred and fourteen eyes of fifty seven children served as the control group (group 2). The mean age was 9.08 (+/-2.82) years with a mean spherical equivalent of -1.90(+/- 1.66) diapers (D) and astigmatism of -0.50 +/- 0.59 D in the case group, compared to the mean age as 9.35 (+/-2.77) years with a mean spherical equivalent of -2.09(+/- 1.67) diapers (D) and astigmatism of -0.55 +/- 0.60 D in the control group. The concentration of atropine eye drops was modified by seasonal variation and used once a day; in general, 0.1% for summers, 0.25% for springs and falls, and 0.5% for winters. Visual acuity, intraocular pressure, and eyeball axial length were evaluated.**

***Results*— At 1-year follow-up, the mean progression 0.28 (+/- 0.75) D of myopia equivalent on atropine treatment group was significantly less than that of the control group 1.23 (+/- 0.44) D. The progression of myopia was significantly correlated with the increases of axial length in cases (r = 0.297, P =0.001) as well as in controls (r = 0.348, P=0.000); however, it was not associated with intraocular pressure (r = -0.0015, P=0.923) among the whole subjects.**

***Conclusion*— After minimizing the photophobia symptoms to improve the medication compliance, the modified use of atropine, based on seasonal variation, is shown promising to slow down the myopia progression for preschool- to school-aged children.**

I. Introduction

Myopia is a common ocular disorder, and high or pathologic myopia (myopia at least −6.0 diapers) is associated with potentially blinding complications, such as macular degeneration, retinal detachment, glaucoma, and cataract.[1-4] The prevalence rate 60% to 80% of myopia in young adults in Taiwan, Hong Kong, and Singapore is higher than those 20% to 50% in the United States and Europe.[1, 5-7] The data released by Taiwanese National Health Bureau in 2003 showed that the prevalence rates of preschool children aged 4-5 years, 5-6 years and 6-7 years were 11.36%, 15.18% and 18.84% respectively. The progression rate, however, became very significant compared to the prevalence data in 1991-1994. At that period, the prevalence rates were only 4.79%, 5.68%, and 7.34% for each age group correspondingly. It appears to be a severe and important national health issue here in Taiwan obviously. The progression rate of myopia is highest in young children, and the average age for stabilization of myopia is approximately 16 years.[1] The earlier onset of myopia, the more rapid progression. Thus, a treatment to slow down or even to arrest the progression of myopia in children can not be overemphasized. The etiopathogeneses of myopia might include genetic basis, excessive accommodation, prolonged near work, and proliferation of chondrocytes on the anterior margins of sclera.[7-11]

Reviewing the current therapeutic modalities used for myopia treatment, atropine

eye drops revealed to retard myopia progression in three randomized clinical trials.[12-14] Three different concentrations of atropine (0.1%, 0.25%, 0.5%) were all shown to have clinically significant effects on controlling myopia progression and 0.5% atropine seemed to be the most effective.[14] However, photophobia and poor medication compliance, especially the highest concentration 0.5% indeed compromised the effort of treatments.[15] We, therefore, hypothesized that different atropine concentration of 0.1%, 0.25%, and 0.5%, based on seasonal variation, sunlight intensity and myopia severity, may provide better physiological adaptation for preschool- and school-aged children who were receiving myopia therapy and lived close to the Tropic of Cancer such as Chia Yi area in Taiwan or in areas of low latitudes.

II. Material and Methods

Study Population

This present study was a university based, case- control study. Two hundred and forty eyes of one hundred and twenty healthy preschool- and school-aged children were recruited from October 2006 to December 2008 in Chiayi area, Taiwan. The case group (group 1) had one hundred and twenty-six eyes of sixty three children; one hundred and fourteen eyes of fifty seven children served as the control group (group 2). Those who had ocular trauma history, traumatic cataract, keratoconus, high myopia (-10 D or

About[1]- Buddhist Tzu Chi General Hospital, Taipei[1]; Chang Gung Memorial Hospital, Chiayi[2]; Harvard Vision Center[3], Taipei, Taiwan.
(Address- 6F., No.276, Alley 3, Lane 219, Sec. 7, Jhongshan N. Rd. Beitou District Taipei 11285 Taiwan Tel:886-922-888-349 Fax:886-2-2877-2519 (e-mail:paulclu@hotmail.com)

higher), high hyperopia, severe astigmatism, ocular hypertension, or glaucoma were excluded.

The cases, aged 4 to 16 years with mean 9.08 (+/-2.82) years, demonstrated a mean spherical equivalent of -1.90(+/-1.66) diopters (D) (range 0 to -7.63 D) and a mean astigmatism of -0.50 +/- 0.60 D (-2.50 to 0 D) (Figure 1a). The controls, aged 4 to 16 years with mean 9.35 (+/-2.77) years, demonstrated a mean spherical equivalent of -2.09(+/-1.67) diopters (D) (range 0 to -7.50 D) and a mean outdoor activities. And progressive spectacles were given for those who have any difficulty in classroom.

III. Study Procedures

All eye drops of concentration 0.1%, 0.25% and 0.5% atropine were commercialized. Initial ocular examinations included uncorrected visual acuity (UCVA), best spectacle corrected visual acuity (BSCVA), intraocular pressure (IOP), and axial length (AL) of eye globe. We repeated those measures at the subjects' on-scheduled visits every four to six weeks. We then collected every three-month data for statistical analysis. One year later, there were one hundred and eighteen eyes completed the study.

IV. Statistical Analysis

Statistical analyses were performed using standard software (SPSS, version 11.01 for Windows, SPSS Chicago, IL). Student's *t* test and correlation coefficient were used for statistical analysis. Ninety-five percent confidence interval

astigmatism of -0.55 +/- 0.60 D (-2.50 to 0 D) (Figure 1b). Study cases were given atropine eye drops once daily for at least 1 year with follow-up. The dose regimen of atropine eye drops including 0.1%, 0.25%, and 0.5% were modified by seasonal variation, sunlight intensity and myopia severity, one drop on each eye every night. In general, the 0.1% concentration was used in summer, the 0.25% dosage in spring and fall, and the 0.5% concentration in winter. Anti-UV sunglasses were prescribed for those who had limits were calculated for differences in mean results. A probability value of $P<0.05$ was considered statistically significant.

V. Results

Refractive Status

Initially, the spherical equivalent of cases ranged from 0 D to –7.63 diopters (D) (average -1.90 +/- 1.66 D) and astigmatism was -2.50 to 0 D (mean -0.50 +/- 0.60 D). After 1 year of follow-up, the mean progression was only 0.28 (+/- 0.75) D of myopia (Table 1, Figure 2a, and Figure 3a). This compares to 1.23 (+/- 0.44) D of the controls (Table 1, Figure 2b, and Figure 3b).and 1.06 (+/- 0.61) D of the national data of general population that did not receive any treatment.16

Table 1 Mean Spherical Equivalent(D

Month	n	Mean	SD	Minimum	Maximum
Cases					
Baseline	126	−1.90	1.66	−7.63	0
3	126	−1.92	1.68	−7.63	0.19
6	126	−1.98	1.84	−8.38	1.25
9	126	−2.04	1.80	−8.38	0.63
12	126	−2.17	1.88	−8.25	0.75
Controls					
Baseline	114	−2.09	1.67	−7.50	-0.250
6	114	−2.86	1.76	−8.50	-1.00
12	114	−3.32	1.79	−8.88	-1.25

SD = standard deviation

VI. Axial Length

Average axial length of cases (group 1) and controls (group 2) at the beginning of the study was 23.78 (+/- 0.94) mm and 23.92 (+/- 0.83) mm respectively. One year later, average axial length of cases (group 1) was 24.12 (+/- 0.99) compared to those of controls 24.78 (+/- 0.96) mm.

Progression of myopia significantly correlated with the increases of axial length for cases (r = 0.297, p=0.001) and for controls (r = 0.348, p=0.000) as shown in Figure 4.

VII. Intraocular pressure

After one-year follow-up the mean intraocular pressure was 18.06(+/- 3.37) and 18.14(+/- 3.29) mmHg of cases and controls respectively, compared to the initial mean intraocular pressure 17.39 (+/- 3.97) and 17.89(+/- 3.48) mmHg. The progression of myopia was not associated with the increase of intraocular pressure among cases (r = 0.0023, p=0.907) nor controls. (r = 0.001, p=0.907) as shown in Figure 5a. The progression of myopia was not associated with intraocular pressure either (r = -0.0015, p=0.923) as demonstrated in Figure 5b.

VIII. Safety

Adverse events like papillae, follicles, visual acuity decreased subjectively, and abnormality of accommodation, in general, were mild in severity. No serious adverse effects were ever reported.

IX. Discussion

Saw reported in an evidence-based review article, that there is no evidence to suggest that bifocal lenses, pressure-lowering eye drops, or soft contact lenses retard the progression of myopia.[16] Only atropine eye drops reveal level B, I. (B = moderately important recommendation; I = strong evidence supporting recommendation) and are very cost effective. Although treatment with 0.5% atropine was the most effective,[14] children and parents usually quit the treatment owing to adverse effects, especially photophobia under strong sunlight clinically. In our series different atropine concentration of 0.1%, 0.25%, and 0.5%, based on seasonal variation and sunlight intensity, mean myopia progression(0.28+/-0.75 diopter per year (D/Y)) was more than 0.5% atropine(0.04 +/-0.63 D/Y), but less than 0.25%(0.45+/-0.55 D/Y) and 0.1% (0.47+/-0.91 D/Y).[14] Thus adjusting the concentration of atropine is highly effective, especially in long and full sunshine area, such as area close to the Tropic of Cancer or areas of low latitudes.

Atropine, a muscarinic antagonist, acts through the mechanism of paralyzing accommodation and direct effect on scleral growth.[17, 18] Our results revealed that progression of myopia was significantly correlated with the increases of axial length. We thus might attribute the retardation of myopia to the slow rate of scleral growth. The adverse effects of atropine such as photophobia, visual acuity decreased subjectively, abnormality of accommodation,[19] macular degeneration, retinal toxicity, and cataract Formation due to excess ultraviolet light exposure were ever reported.[20] We prescribed anti-UV sunglasses or suggested a sunscreen hat wearing for patients having outdoor activities.

Siatkowski from US Pirenzepine Study Group and Tan from Asian Pirenzepine Study Group reported that 2% pirenzepine ophthalmic gel, a subtype selective M1 anti-muscarinic antagonist, used twice daily had a mean increase in myopia of 0.26 D and 0.47 D respectively over a 1-year treatment period. Their placebo-controlled groups revealed a mean increase in myopia of 0.53 D and 0.84 D correspondingly.[21, 22]

Chua in Singapore used 1% atropine to treat myopia and reported in year 2006 that myopia progression was only -0.28+/-0.92 D in treated group compared to -1.20+/-0.69 D/Y in placebo-controlled group;[23] Fan in Hon Kong also used topical 1% atropine eye ointment to treat myopia and get the similar result that myopic progression was significantly less (P = 0.005) in the atropine group (+0.06 +/- 0.79 D) than in the control group (-1.19 +/- 2.48 D). [24] However, most children or their parents will quit the treatment in summer if using high concentrated atropine e.g. 0.5% or 1%.

Previous study about pressure-lowering eye drops showed no effect of retarding myopia progression.[25] We questioned that weather increased intra-ocular pressure (IOP) could affect myopia progression or not. In this study, the result showed that progression of myopia was not associated with IOP (Figure 5). We try to understand the reason why subjects gave up the therapy in the middle of treatment. There were some reasons reported such as irritation from eye drop, over expectation of myopia control, misunderstanding or incomplete children and parental education, and too much time spent on ocular examinations.

By providing a better physiologic adaptation, we should be able to improve treatment. To improve the compliance, the frequency of installation reduced to twice a week for those very low myopes (-0.75 to 0 D). Not only were anti-UV sunglasses prescribed for those who had outdoor activities, but progressive spectacles were also given for those who have any difficulty in classroom or in reading. The trifocal lenses, not bifocal, had a better cosmetic appearance and preference for children. For those low to moderate myopes, atropine appears to retard the progression of myopia promisingly. Whether pathologic or extreme myopia could be prevented by the use of atropine or not still needs a large scale and longitudinal study.

It still remains a myth that some cases resulted in much improvement of myopia or much reduction of myopic power, for example myopia of -1.25 D back to ammetropia (0 to +0.25 D) or -2.50 D back to -1.0 D, after three- to six-month atropine treatment and became stabilized afterwards; however, it did not reveal much reduction of myopic power among controls. The axial length appeared only minimal increase after continuous atropine therapy for more than one year. The effect was attributed partially to scleral rigidity or slower scleral growth. We thus have to reconsider the

phenomenon of pseudomyopia that the power difference before and after pupil dilatation in clinic, because the mydriatic medication might not release or paralyze the accommodative power completely. More cases are needed for further analysis. We hypothesize the "trigger theory" that children born by high myopic parents were like a pistol loaded with bullets (genome) that might not do any harm if they were not triggered (risk factors); a lot of damages (complications) followed if the triggers are pulled again and again. Atropine acted as a viscous medium or damper to slow down that bullet thus reduce the damage. In Singapore, Tong et al. reported that even after stopping treatment, eyes treated with atropine demonstrated higher rates of myopia progression compared with eyes treated with placebo. However, the absolute myopia progression after 3 years was significantly lower in the atropine group compared with placebo.[26] We thus strongly suggest that myopia should be treated intensively as early as possible in order to avoid the late complications caused by high myopia. By improving the medication compliance and reducing the complaints of photophobia, the modified use of atropine concentration of 0.1%, 0.25%, and 0.5%, based on seasonal variation and myopic severity, could effectively slow down the myopia progression in preschool- and school-aged children.

X. References

1) Tan NW, Saw SM, Lam DS, Cheng HM, Rajan U, Chew SJ. Temporal variations in myopia progression in Singaporean children within an academic year. Optom Vis Sci 2000;77:465-472.
2) Mitchell P, Hourihan F, Sandbach J, Wang JJ. The relationship between glaucoma and myopia: the Blue Mountains Eye Study. Ophthalmology 1999;106:2010-2015.
3) Pierro L, Camesasca FI, Mischi M, Brancato R. Peripheral retinal changes and axial myopia. Retina 1992;12:12-17.
4) Fan DS, Lam DS, Lam RF, Lau JT, Cheung EY, Lai RY, Chew SJ. Prevalence, Incidence, and Progression of Myopia of School Children in Hong Kong. Invest Ophthalmol Vis Sci 2004;45:1071-1075.
5) Lin LL, Shin YF, Tsai CB, Chen CJ, Lee LA, Hou PK. Epidemiologic study of ocular refraction among schoolchildren in Taiwan in 1995. Optom Vis Sci 1999;76:275-281.
6) Katz J, Tielsch JM, Sommer A. Prevalence and risk factors for refractive errors in an adult inner city population. Invest Ophthalmol Vis Sci 1997;38:334-340.
7) Saw SM, Katz J, Schein OD, Chew SJ, Chan TK. Epidemiology of myopia. Epidemiol Rev 1996;18:175-187.
8) Chen JC, Schmid KL, Brown B. The autonomic control of accommodation and implications for human myopia development: a review. Ophthalmic Physiol Opt 2003;23:401-422.
9) Yap M, Wu M, Liu ZM, Lee FL. and Wang SH. Role of heredity in the genesis of myopia. Ophthalmic Physiol Opt 1993;13:316-319.
10) Farbrother JE, Kirov G, Owen MJ, Pong-Wong R, Haley CS, Guggenheim JA. Linkage Analysis of the Genetic Loci for High Myopia on 18p, 12q, and 17q in 51 U.K. Families. Invest Ophthalmol Vis Sci 2004;45:2879-2885.
11) Wang IJ. Shin YF. The effect of intravitreal injection of atropine on the proliferation of scleral. J Ocul Pharmacol Ther 1998;14:337-343.
12) Bedrossian RH . The effect of atropine on myopia. Ophthalmology1979;86:713-717.
13) Chou AC, Shih YF, Ho TC, Lin LLK. The effectiveness of 0.5% atropine in controlling high myopia in children. J Ocul Pharmacol Ther 1997;13:61-67.
14) Shih YF. Chen CH. Effects of different concentrations of atropine on controlling myopia in myopic children. J Ocul Pharmacol Ther 1999;15:85-90.
15) Chiang MF, Kouzis A, Pointer RW, Repka MX. Treatment of childhood myopia with atropine eyedrops and bifocal spectacles. Binocul Vis Strabismus Q 2001;16:209-215.
16) Saw SM, Shin-Yen EC, Koh A, Tan D. Interventions to Retard Myopia Progression in Children. Ophthalmology 2002;109:415-421.
17) Tigges M, Iuvone PM, Fernandes A, Sugure MF, Mallorga PJ, Laties AM, Stone RA Effects of muscarinic cholinergic receptor antagonists on postnatal eye growth of rhesus monkeys. Optom Vis Sci 1999;76:397-407.
18) Lind GJ, Chew SJ, Marzani D, Wallman J. Muscarinic acetylcholine receptor antagonists inhibit chick scleral chondrocytes. Invest Ophthalmol Vis Sci 1998;39:2217-2231
19) Goss DA. Attempts to reduce the rate of increase of myopia in young people - a critical literature review. Am J Optom Physiol Opt 1982;59:828-841.
20) Luu C, Lau AM, Koh AH, Tan D. Multifocal electroretinogram in children on atropine treatment for myopia. Br J Ophthalmol 2005;89:151-153.
21) Siatkowski RM, Cotter S, Miller JM, Scher CA, Crockett RS, Novack GD. US Pirenzepine Study Group. Safety and efficacy of 2% pirenzepine ophthalmic gel in children with myopia: a 1-year, multicenter, double-masked, placebo-controlled parallel study. Arch Ophthalmol 2004;122:1667-1674.
22) Tan DT, Lam DS, Chua WH, Shu-Ping DF, Crockett RS; Asian Pirenzepine Study Group. One-year multicenter, double-masked, placebo-controlled, parallel safety and efficacy study of 2% pirenzepine ophthalmic gel in children with myopia. Ophthalmology 2005;112:84-91.

23)
24) Chua WH, Balakrishnan V, Chan YH, Tong L, Ling Y, Quah BL, Tan D.

26) Fan DS, Lam DS, Chan CK, Fan AH, Cheung EY, Rao SK. Topical atropine in retarding myopic progression and axial length growth in children with moderate to severe myopia: a pilot study. Jpn J Ophthalmol. 2007;51:27-33.
27) Jensen H. Timolol maleate in the control of myopia. A preliminary report Acta Ophthalmol (Suppl) 1988:128-129.
28) Tong L, Huang XL, Koh AL, Zhang X, Tan DT, Chua WH. Atropine for the treatment of childhood myopia: effect on myopia progression after cessation of atropine. Ophthalmology. 2009;116:572-579.

25) Atropine for the treatment of childhood myopia. Ophthalmology. 2006 ;113:2285-2291.

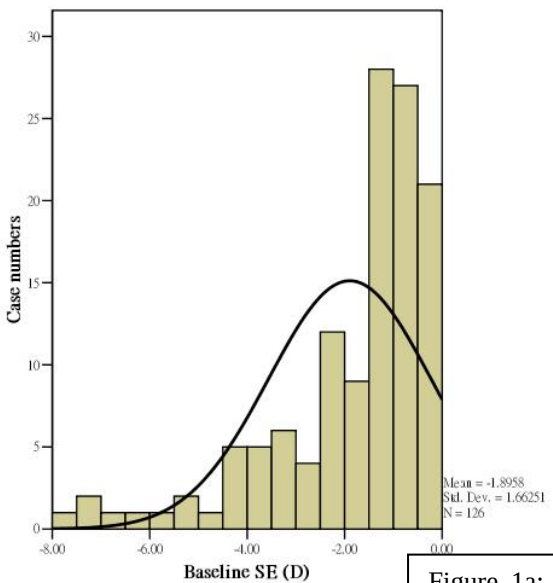


Figure 1a: Cases (Group 1)

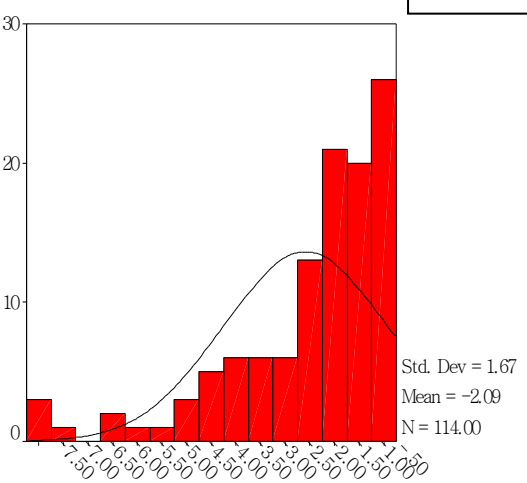


Figure 1b: Controls (Group 2)

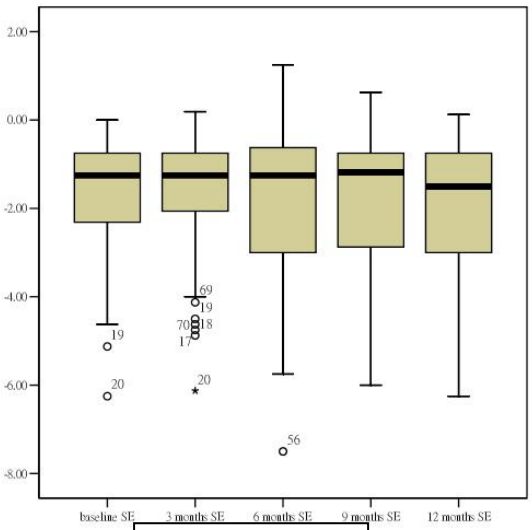


Figure1b: Controls (Group 2)

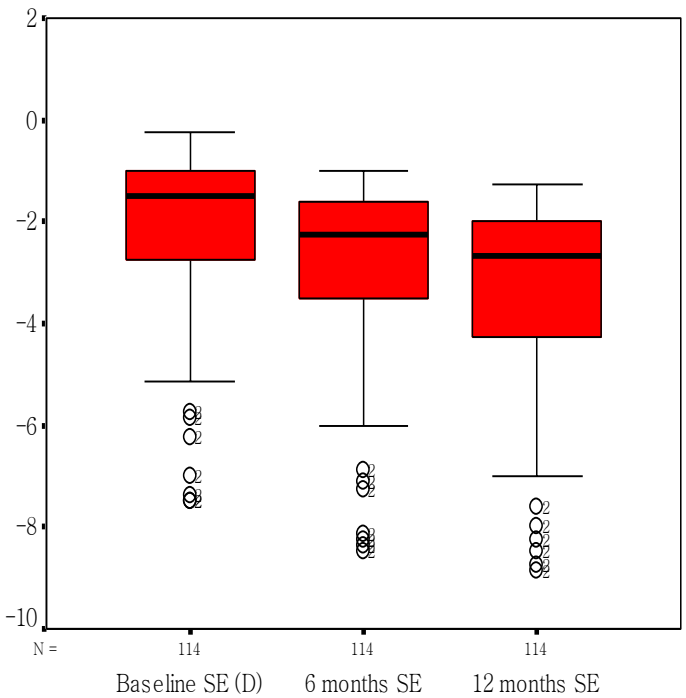


Figure 2b

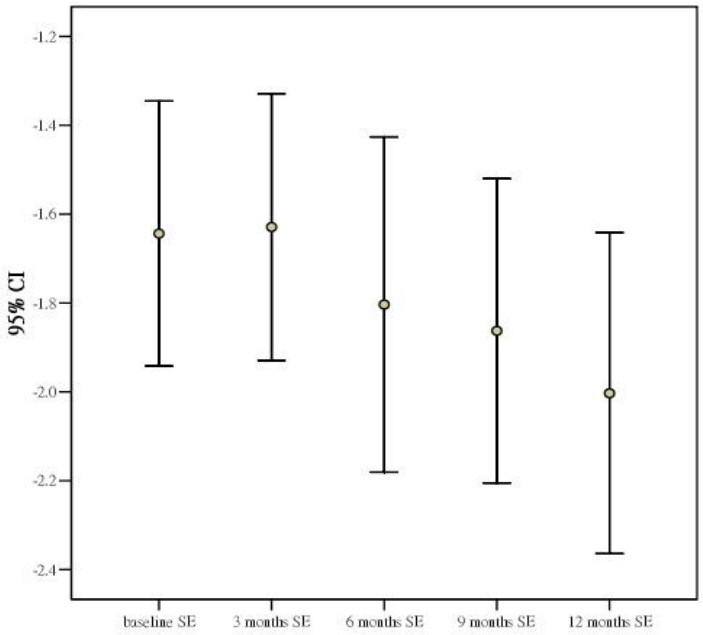


Figure 3a

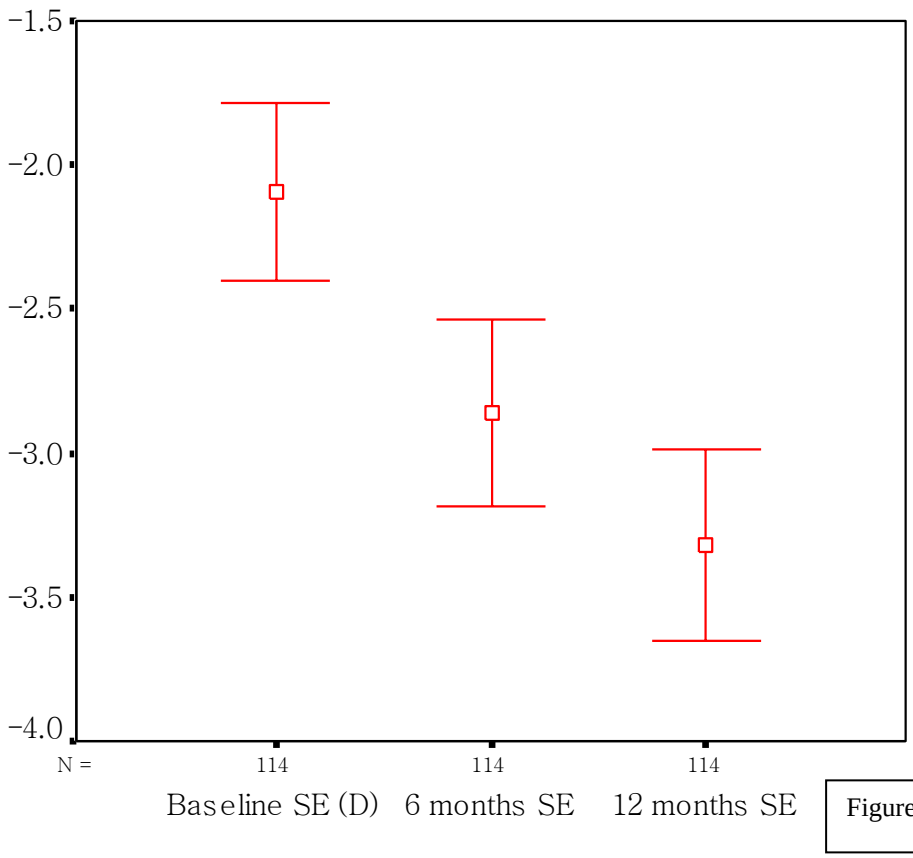


Figure 3b

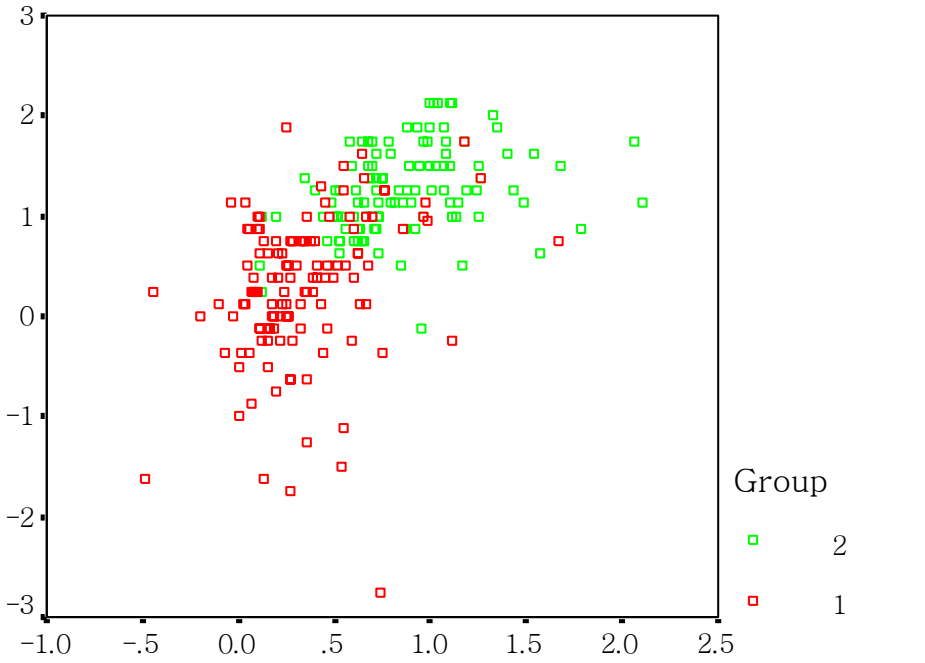


Figure 4

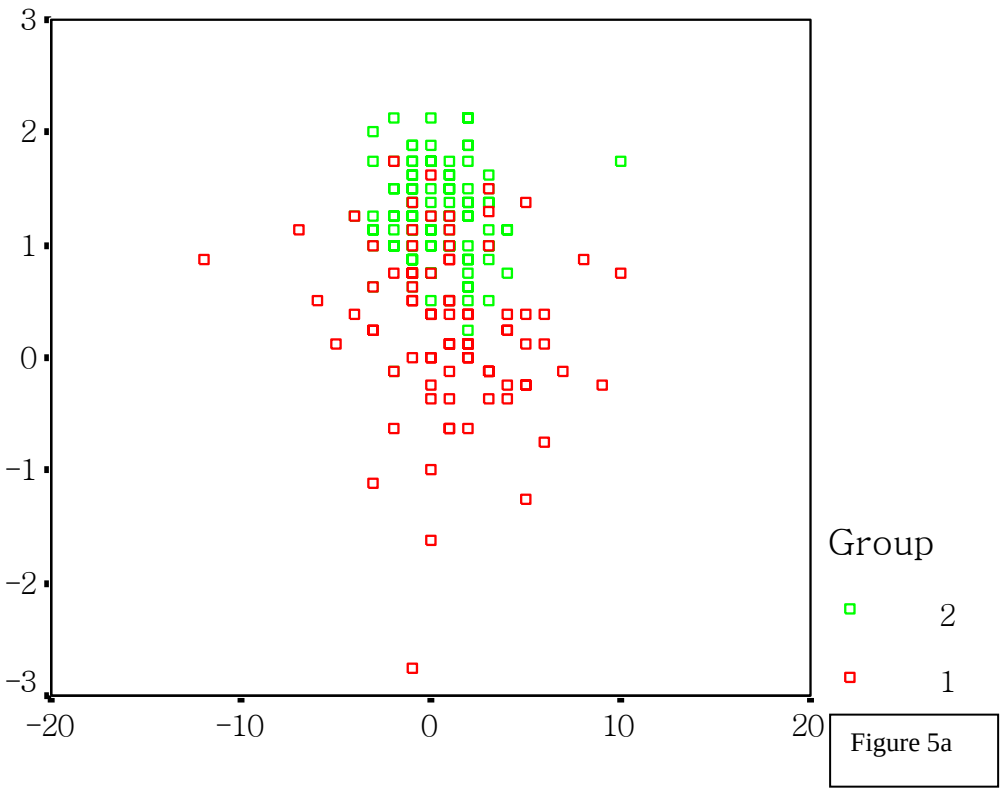


Figure 5a

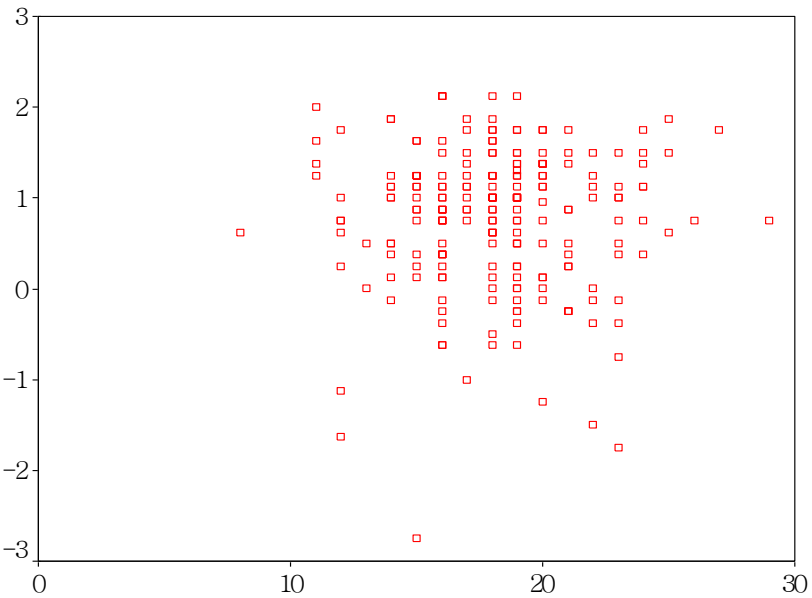


Figure 5b

XI. Figure Legends

1) Figure 1a. The distribution of myopic status of one hundred and twenty six eyes of cases (group 1) at the study entry.

2) Figure 1b. The distribution of myopic status of one hundred and fourteen eyes of controls (group 2) at the study entry.

3) Figure 2a. The spherical equivalent (SE) of baseline, three-month follow-up, six-month follow-up, nine-month follow-up, and one-year follow-up of cases (group 1).

4) Figure 2b. The spherical equivalent (SE) of baseline, six-month follow-up, and one-year follow-up of controls (group 2).

5) Figure 3a. The 95% confidence interval (CI) of baseline, three-month follow-up, six-month follow-up, nine-month follow-up, and one-year follow-up.

6) Figure 3b. The 95% confidence interval (CI) of baseline, six-month follow-up, and one-year follow-up.

7) Figure 4. The progression of myopia was significantly correlated with the increases of axial length in cases (L1: r = 0.297, P =0.001) and in controls (L2: r = 0.348, P=0.000).

8) Figure 5a. The progression of myopia was not associated with the increase of intraocular pressure in cases (L1: r = 0.0023, P=0.907) nor in in controls (L2: r = 0.011, P=0.907).

9) Figure 5b. The progression of myopia was not associated with intraocular pressure (r = -0.0015, P=0.923) among the whole subjects.

Late Psychological Impacts of Wartime Low Level Exposure to Sulfur Mustard On Civilian Population Of *Direh* (17 Years After Exposure)

Running head: Mental health of Low level Sulfur Mustard exposed

Falahati Farahnaz[1] Khateri Shahriar[2] Soroush Mohammad Reza[3]

Amirali Salamat[4]

GJMR Classification (FOR)
380100,321000,321021,321217

***Abstract*- In the present study a retrospective cohort was conducted among a total of 460 civilians who were exposed to low level Sulfur Mustard in the Iranian village of Direh, evaluating the prevalence of symptoms of depression, anxiety, and PTSD which are known to be the most common adverse mental health consequences of war. Subjects were administered the Beck Depression Inventory and the Hamilton Rating Scale for Anxiety while the Clinician-Administered PTSD Scale (CAPS) was used among 80 of this population to evaluate PTSD symptoms.**

The results showed that the exposure to low level of Sulfur Mustard among civilians is associated with higher prevalence of depressive, anxiety and PTSD symptoms. These results reiterated the need for provision of psychiatric support for these populations.

Keywords: Sulfur Mustard, low level exposure, Iran-Iraq war, mental health impacts, civilian

I. Introduction

The use of chemical weapons by Iraqi forces against Iran during the 1980-88 Iran-Iraq conflict was a horrifying epic in the annals of modern warfare, inflicting enormous suffering that continues to the present day in the form of latent illness among surviving victims of chemical attack, who number approximately 45,000 at the time of this writing (Khateri 2005). Toxic agents used by the Iraqis fell into two major categories based on chemical composition and casualty-producing effects: One class of weapons used were organophosphate neurotoxins, primarily Tabun and Sarin, which inhibit essential enzymes necessary for nerve transmission, causing death as a result of failure to maintain control of major organ systems, particularly the lungs. "Mustards", which are sulfur or nitrogen-based vesicant agents that degrade tissue of exposed personnel causing severe chemical burns were also used extensively. In this category, sulfur mustard (bis (2-chloroethyl) sulphide, SM) became a highly favored combat multiplier due to its high stability, ease of manufacture and terrifying effects. SM was used extensively both against Iranian troops and unprotected civilians, which included substantial numbers ofIraqi Kurds (United Nations Documents 1988) Surviving victims suffer from a diverse range of chronic illnesses, witHaines 2003).

Elevated rates of mental health problems have also been documented in Iranian chemical attack victims (Hashemian , Khoshnood, Desai, Falahati, Kasl, Southwick ,2006), a result consistent with previous studies demonstrating positive correlation between the length and severity of exposure to high stress environments and prevalence of various forms of chronic psychological distress (Fazel, Wheeler, Danesh 2005). Some evidence that the physical and mental trauma of SM exposure engenders particularly severe psychological consequences was described in reports of the mental health status of American military personnel exposed to the agent in classified experiments during the 1940s. Approximately half of these subjects were subsequently diagnosed with post-traumatic stress disorder (PTSD) which in many cases became lifetime conditions (Schnurr, Friedman, Green 1996), (Schnurr, Ford, Friedman, Green, Dain, Sengupta 2000). Neuropsychiatric evaluation of 1428 Iranian veterans, 3-9 years after exposure to SM demonstrated conditions such as anxiety (15%), depression (46%) and personality disorders (31%) to exist among the subjects (Balali 1992). These results notwithstanding, very little effort has been made to characterize the psychological impact of exposure to chemical warfare agents. A potentially landmark study in this respect, was a cross-sectional randomized survey conducted in 2004 by Janbazan Medical and Engineering Research Center (JMERC), which compared prevalence of psychological distress among residents of three Iranian communities: one which had been subjected to low-intensity conventional warfare; another which had sustained high-intensity conventional warfare; and a third town that had been subjected to high-intensity conventional warfare and an air-delivered SM attack (Hashemian et al). As expected, the highest rates of persistent psychological disorders were observed among residents of the community which had sustained both high-intensity combat and mustard exposure (Hashemian, et al). These results are predicted in part by the experience of SM-exposed U.S, servicemen (Schnurr, et al), who also experienced severe psychological impacts. However, a major question that remains unanswered in studies conducted thus far is whether a minimum threshold

About1-Janabazan medical & engineering research center (e-mail: f.falahati@gmail.com):19615/616 Tehran – Iran)

of SM exposure exists, above which, increasingly severe impact on mental health may be expected. To address this question and extend the results of the aforementioned 2004 JMERC study, residents of Direh, a Kurdish village in the western border of Iran was evaluated for occurrence of depression, anxiety, and PTSD. In 1988 this community was in the vapor plume of attacks with air-delivered SM munitions, however the town did not sustain direct hits and its residents were therefore subjected to substantially lower concentrations of SM than the population of Sardasht, the Kurdish town attacked with both conventional and chemical munitions described in the aforementioned 2004 JMERC study (Hashemian, et al). In the present study it is hypothesized that late-occurring mental health effects are prevalent among individuals exposed to "low" concentrations of SM at rates significantly in excess of those in a non-exposed control population; but lower than in populations exposed to "high" SM doses. Here "low" and "high" are subjective terms referring to relative presumed exposure to unprotected civilians based on proximity to the site of impact of SM-containing munitions. It is expected that the results of this investigation, when considered in context with the Sardasht report (Hashemian, et al), will allow improvement in the accuracy with which a person may be classed as "exposed" or "unexposed". Specifically, this work is expected to substantially improve ongoing efforts to define "low dose" exposures and correlate them with known physical and psychological effects.

II. Material & Methods

A retrospective cohort study was conducted among civilians of two villages of western part of Iran. subjects were 540 people who are living in a western countryside of Iran, (Direh and a nearby village Khorata) both villages have been exposed to war violence and were occupied during the Iran-Iraq war, all were over 18 and 460 of them were exposed to low level of Mustard gas in Direh village (cases), the others (80) were not gassed but engaged in war (controls). Subjects were briefed on the main features and objectives of the project before they voluntarily participated in completing the questionnaires while adequate and appropriate privacy was provided and confidentiality of information ensured.Exposure to sulfur mustard was documented by their medical records filed at Janbazan organization (Veterans Affair).A randomized cluster sampling was conducted among both cases and controls, each cluster comprising 20 households. All cases were selected by a randomized cluster selection from all parts of the village with each subject at least 1 house away from the other, and in every house one victim selected randomly. All face to face interviews were conducted in Farsi by the first author who had received formal training in each of the instruments 4 questionnaires were used for every subject as follows:

Demographic questionnaire including age, sex, educational status, marital status, employment; Beck Depression Inventory for depression (Beck, Beck 1972),Clinician-Administered PTSD scale (CAPS) (Blake, Weathers, Nagy 1995) and Hamilton Anxiety Rating Scale for anxiety) (Hamilton 1969).

We used the self report short listing Beck depression Inventory (consisting of 13 items) which evaluated existence and severity of depression symptoms against the DSM-IV criteria. The questionnaires were translated to Farsi with their validity and reliability having been approved.

All data were gathered by a general practitioner interviewer and approximately 60 minutes was allocated to completing all questionnaires.

Depressive symptoms were categorized as none (0-5), mild (6-9), moderate (10-15) and severe (≥ 16)

Anxiety symptoms were categorized as none (0-5), mild (6-9), moderate (10-14) and severe (≥15)

Using CAPS, current and lifetime PTSD were evaluated

Data analysis:

Prevalence rates for symptoms of Anxiety, depression and PTSD and other characteristics were calculated and analyzed using statistical software (SPSS, version 12, spss Inc, Chicago III); the severity of PTSD, anxiety and depressive symptoms was analyzed using ✗ T tests to evaluate differences in categorical variables and T tests to evaluate differences in continuous variables, if continuous variables were not normally distributed, nonparametric tests were applied. The *P l*evel for statistical significance was set at 0.05.

Hamilton Anxiety rating which consists of 14 items was used to rate the severity of anxiety.

And finally CAPS and its five associated features which is a structured clinical interview to screen adults for symptoms of PTSD against the DSM_IV criteria, was used to evaluate the severity of existing PTSD symptoms or its history in the past.

III. Results

Among Mustard gas exposed victims of Direh village 460 of them were evaluated by Hamilton anxiety rating and Beck depression inventory and 80 of them were interviewed by CAPS, all controls were also interviewed by the same questionnaire. The participation rate was 100% for both of case and control groups. Overall, the mean age of participants was 35 years (SD, 11.0)

All had Kurdish background; Most of them were of little education, married (59.5%) and men (53%).Characteristics of both groups were listed in Table 1.

.Mild, moderate and severe symptoms of anxiety were significantly higher in the SM exposed population (P Value<.05) (See table 2) Moderate degree of anxiety is the commonest form of symptoms in all age groups.

Symptoms of anxiety (mild, moderate and severe) are seen in 95.4 % of cases and 29.5% of controls

The mean score of anxiety was higher in cases (33.7 Vs 16.19), the mean of anxiety in different Aging groups is also significantly different (P Value<.05).

The mean score of anxiety was lower in employed persons in cases and controls and despite the higher mean of anxiety score in women, occupation and gender didn't have any significant effect on decreasing anxiety score.

The mean anxiety score in singles was significantly lower than the married ones, (P Value<.05)
The disability percentage also had a significant effect on anxiety symptoms among the cases
(P Value<.05).
In evaluation of depressive symptoms 59.1 % of controls and 98.7 % of cases were observed to have depressive symptoms. (See table 2)
Prevalence of Mild form of depression wasn't significantly different in the 2 groups but moderate and severe forms of depression were more common in the SM exposed population. (P Value<.05)
Despite the lower mean of depressive symptoms in the lower end of age groups, the effect of age wasn't significant on cases and controls.
Depressive scores were higher in women nonetheless; gender variation wasn't significant in both groups.
There was a significant correlation between the disability rate and the mean depressive score of cases. (P Value<.05)
Evaluation of PTSD symptoms revealed that as with other mental conditions, the SM exposed population had significantly higher lifetime susceptibility to PTSD than the controls (P Value<.05) (table 3)

IV. Conclusion

Depression, anxiety, and PTSD are common in war afflicted countries which can be put down to unusual stressors such as life threat, scenes of shelling, being shot at or displaced, losing a loved one or family members, occupation of home town (as in the case of this study), being arrested and in this case exposure to low levels of Sulfur Mustard.
The summative rate of mental disorders in Iran is reported at 11.9% to 23.8% by different authors (Noorbala, Mohammad, and Yasami 2001). This study demonstrates higher than normal population rates of mental symptoms in our control group; this study may have encompassed different prospective and employed different methods and classification. However, some previous studies have already demonstrated a higher rate in rural populations as compared to city dwellers (Noorbala, Bagheri Yazdi, Yasami, 2004).
Post traumatic stress disorder (PTSD) may develop due to exposure to an extraordinary stressful trauma and is a relatively common finding in community based epidemiologic surveys (5-6%for men and 10-12% for women) (Magruder, Frueh, Knapp, 2005).
The use of chemical weapons added to the human cost of the rather prolonged Iraq-Iran war. However among the considerable amount of government or NGO backed studies conducted in this area, few, (if any) have addressed the long term psychological impacts of chemical warfare on civilian populations. To this end, any attempt directed at the betterment of the mental health care of the affected people seems to be of priority and yield dramatic results. This study could also raise the need for research on other types of mental disorders such as personality disorders among the chemical exposed populations. Follow-up of exposed human subjects could provide a wealth of information which could serve as a basis for their health support. Based on the results of this study it may be elicited that more financial resources should be allocated to educational support programs, psychological support and psychotherapy methods, improvement of active coping behaviors, interaction on events and social support for both family and the community to reduce the untoward impacts.
The results of this study gave us an idea of the long term health effects of low level SM exposure among a civilian population in the western part of Iran 17 years after the event. We realized that exposure to chemical weapons among civilians is associated with a higher prevalence of depressive symptoms and anxiety disorders as compared to those exposed to war related violence and occupation alone.
Also the results indicated that this effect relates to the degree of disability due to exposure.
There were some limitations to this study, first was difficulty of our subjects in recalling traumatic events and psychological symptoms that they experienced at the time of exposure to traumatic events, the second limitation was failing to exclude people with a positive history of somatic conditions that predispose people to psychiatric symptoms including anemia and thyrotoxicosis which are common medical conditions in Iran.
Notwithstanding the limitations, the results of this study also were in line with earlier epidemiologic studies in showing higher prevalence of mental disorders among the illiterate, semiliterate and married individuals in Iran (Noorbala, Mohammad, Bagheri Yazdi, 1988) This could perhaps be due to the fear of an unusual warfare, less known and with less tangible physical injuries among the less educated people and the burden of family obligations amongst the married.

V. Comment

Exposure to chemical warfare agents is an extraordinary stress that may cause serious mental adverse effects even at low level doses
Educational support programs, psychological support and psychotherapy methods may improve coping behaviors and reduced the impact of such events.

VI. References

1) Beck AT, Beck RW (Dec 1972). Screening depressed patients in family practice, a rapid technic. Postgrad Med, 52(6):81-5
2) Blake DD, Weathers FW, Nagy LM, Kaloupek DG, Gusman FD, Charny DS, Keane TM (Jan 1995), The development of the Clinician-Administered PTSD Scale. J. Trauma. Stress, 8(1):75-90
3) Balali M (1992, pp 15-37). Evaluation of late toxic effects of Sulfur Mustard poisoning in 1428 Iranian veterans, in: Proceedings of the Seminar on Late Complications of Chemical Warfare Agents in Iranian Veterans, Veteran Foundation, Tehran, Iran..
4) Fazel M, Wheeler J, Danesh J (2005). Prevalence of serious mental disorder in 7000 refugees

5) resettled in western countries. Lancet, 365:1309-1314
7) Hashemian F, Khoshnood K, Desai MM, Falahati F, Kasl S, Southwick S (2006 Aug 2) Anxiety, depression, and posttraumatic stress in Iranian
8) survivors of chemical warfare, JAMA, 296(5):560-6
9) Khateri S, Ghanei M, Keshavarz S, Soroush M, Haines D(2003).Incidence of lung, eye, and skin lesions as late complications in 34,000 Iranians with wartime exposure to mustard agent. J Occup Environ Med; 45:1136-1143
10) Khateri SH (2005).Victims of Chemical weapons in Iran. Society for chemical weapons victims support (SCWVS), Tehran: Aftab –e Graphic,
11) Magruder K M, Frueh BC, Knapp RG, Davis L, Hamner M B, Hebert R , Gold P B, Arana GW (May-Jun 2005)" Prevalence of Post traumatic stress disorder in veterans affair primary care clinic. Gen Hosp. Psychiatry; 27(3):169-79
12) Noorbala A A, Bagheri Yazdi S A, Yasami MT, Mohammad K (2004).Mental health survey in adult population in Iran. Br J Psychiatry (184):70-73
13) Noorbala A A, Mohammad K, Bagheri Yazdi S A (1998). Survey of Psychiatric disorders in Tehran city, Hakim Magazine (5):101-110
14) Noorbala A A, Mohammad K, Yasami MT, Mohammad K (2001). A view of mental health in Iran. Tehran: Iranian Red Crescent Society Publication
6) Hamilton A (1969).Diagnosis and Rating of Anxiety. Br J Psychiatry(3):76-79
15) Schnurr PP, Ford JD, Friedman MJ, Green BL, Dain BJ, Sengupta A(2000). Predictors and outcomes of posttraumatic stress disorder in World War II veterans exposed to mustard gas. J Consult Clin Psychol; 68:258-268)
16) Schnurr PP, Friedman MJ, Green BL (1996). Post Traumatic Stress Disorder among World War II mustard gas test participants. Mil Med; 161:131-6.
17) United Nations Security Council (1988 April 25).Report of the mission dispatched by the Secretary General to investigate allegations of the use of chemical weapons in the conflict between the Islamic Republic of Iran and Iraq. , S/19823/ Addendum 1.New York, USA United Nations.
18) United Nations Security Council (1988 July 25), Report of the mission dispatched by the Secretary General to investigate allegations of the use of chemical weapons in the conflict between the Islamic Republic of Iran and Iraq. S/ 20063/ Addendum 1. New York, USA, United Nations

Table 1-Demographic characteristics of study groups

Study groups / Demographic characteristics		Low level SM exposed group(N= 460) Number /Percent	Non SM exposed group(N= 80) Number /Percent
Age/y	<30	206(44.79)	33(41.25)
	31-59	230(50)	40(50)
	>60	24(5.21)	7(8.75)
Marital status	single	182(39.7)	30(37.5)
	Married	278(60.2)	50(62.5)
Sex	male	215(46.73)	34(42.5)
	female	245(53.26)	47(57.5)

Table2: Prevalence of Anxiety and Depressive symptoms in the study groups

groups / symptoms		Low level SM exposed (N= *460*) Number /Percent	Non SM exposed (N=*80*) Number /Percent
Anxiety	*mild*	44 (9.6)	4(5.2)
	Moderate	308 (67.0)	18(23.4)
	Severe	87 (18.9)	1(1.3)
	None	21 (4.5)	57(70/1)
Depression	mild	148 (32.8)	25 (32.5)
	Moderate	250(55.4)	13 (16.9)
	Severe	47(10.4)	2 (2.6)
	None	15 (1.4)	40 (48)

Table3: Prevalence of PTSD in the study groups

groups / PTSD	cases (N=*80*) Number/Percent	Controls (N=*80*) Number /Percent
Current	1(1.3)	5(6.5)
Lifetime	31(39.2)	4 (5.2)
None	48(59.5)	71(88.3)

The Insulin Bio Code - Prima sequences

Lutvo Kuric

GJMR Classification (FOR)
320305.321011.321006

Abstract- **The subject of the research we are discussing in this text is the cyber-information access to the research of the amino acidic constitution of insulin. Strictly speaking, the subject of this research is finding of an adequate scientific language that could describe this phenomenon, study of the genetic information, as well as relationship between the genetic language of the protein and the theory of the system and cybernetics. The result of the research that we have carried out clearly shows that there is a matrix code in insulin. It also shows that the coding system within the amino acidic language gives a full information, not only for the amino acid „record", but also for its structure, configuration and its various shapes. In the following text we shall discuss the issue of the existence of the insulin code, and also the issue of coding of individual structural levels in this protein.**

Insulin is composed of amino acids with various numerical values. This numerical values are in an irregular order. For example, the first one has 10 atoms, the second one 22. Their frequency is (+)12. Second amino acid has 22 atoms, and the third one 19. Their frequency is (-)3; etc. Frequency is the measurement for establishment of intervals of numerical values of amino acids in proteins. This value can be positive, negative or a zero value. These frequencies are showing us one completely new dimension of protein sequencing. Through these frequencies we can establish which of amino acids are of primary, and which are of secondary significance in biochemical processes of insulin.

This paper reports the discovery of new methods for developing the new technologies in genetics. It is about the most advanced digital technology which is based on program, cybernetics and informational systems and laws. The results in the practical application of the new technology could be useful in medicine, bioinformatics, genetics, biochemistry, and other natural sciences.

Keywords-Human Insulin, Frequencies of Insulin, Bio code, Genetics Code; Amino acids Code; Evolution

I. Introduction

Biological specificum of protein, its place and role in life processes depends on the positioning of amino acids in its molecules. The molecule of insulin we can understand as words built from letters, i.e. amino acids. The meaning of words is determined by positioning of letters. Each of these words has its biochemical base. If this base is expressed by corresponding discrete numbers, we find out that the base has its own program, cybernetic and information characteristics. In fact, we will find out that the sequencing of the molecule is conditioned and determined not only by biochemical, but also by cybernetic and information principles. For this reason, in this research we will deal more with quantitative, and less with qualitative (Characteristics of the genetic information and its biochemical foundation. To that effect we will select certain physical and chemical factors in order to express the given genetic information. We will than attribute certain numeric values to these factors by which we will measure and express these factors. In this way we will discover the connection, if the connection really exists, between the quantitative ratios in the process of transfer of genetic information and qualitative appearance of the molecule of insulin. How shall we select these factors? We shall give preference to classical physical and chemical parameters, such as: number of atoms in amino acids, their analogue values, and position of amino acids in the peptide chain, frequencies and other parameters. There is a rather large number of these parameters, and each of them gives some genetic information in a certain way. Once we do this, we shall find out that indeed there is a mathematical connection between quantitative ratios and qualitative appearance of the genetic processes' biochemistry. In fact, we will find out that there is a measurement we can use to express the biochemistry of insulin. There are discrete codes that can show us one radical new dimension of the genetic process functioning. And in that dimension we can find an explanation for the given empirical reality

About-Independent Researcher, Bosnia and Herzegovina, 72290 Novi Travnik, Kalinska 7
(Phone : 00387 61 763 917,Email : lutvokuric@yahoo.com)

II. Methods

The sample of an insulin can be represented by two different forms: one is the discrete form and the other is the sequential form. In the discrete form, a insulin is represented by a set of discrete codes or a multiple dimension vector. In the sequential form, insulin is represent by a series of amino acids according to the order of their position in the chains 1AI0. Therefore, the sequential form can naturally reflect all the information about the sequence order and length of Insulin. The crux is: can we develop a different discrete form to represent insulin that will allow accommodation of partial, if not all, sequence-order information? Since a protein sequence is usually represented by a series of amino acid codes, what kind of numerical values should be assigned to these codes in order to optimally convert the sequence-order information into a series of numbers for the discrete form representation?

How functioning of biochemistry is determined through cybernetic information principles, will be discussed further in next section.

III. Results

We shall now give some mathematical evidences that will prove that in the biochemistry of insulin there really is programmatic and cybernetic algorithm in which it is „recorded", in the language of mathematics, how the

molecule will be built and what will be the quantitative characteristics of the given genetic information.

IV. Expression of Insulin Frenquencies

The matrix mechanism of Insulin, the evolution of biomacromolecules and, especially, the biochemical evolution of Insulin language, have been analyzed by the application of cybernetic methods, information theory and system theory, respectively. The primary structure of a molecule of Insulin is the exact specification of its atomic composition and the chemical bonds connecting those atoms.**R6 INSULIN HEXAMER (D1AI02)**

The structure 1AI0 has in total 12 chains. Out of these 2 are sequence-uniqueIdentical chains BDFHJL and A,C,E,G,I,K.

Number of atoms

F	V	N	Q	H	I	C	G	S	H
23	19	17	20	20	22	14	10	14	20
1	2	3	4	5	6	7	8	9	10

L	V	E	A	L	Y	L	V	C	G
22	19	19	13	22	24	22	19	14	10
11	12	13	14	15	16	17	18	19	20

E	R	G	F	I	Y	T	…	T
19	26	10	23	22	24	17	…	17

21 22 23 24 25 26 27 … 306

Rank = 1,2,3..., 306.

Aforementioned aminoacids are positioned from number 1 to 30. Numbers 1, 2, 3, n... present the position of a certain aminoacid. This positioning is of the key importance for understanding of programmatic, cybernetic and information principles in this protein. The scientific key for interpretation of bio chemical processes is the same for insulin and as well as for the other proteins and other sequences in biochemistry. The first aminoacid in this example has 23 atoms, the second one 19, the third one 17, etc... Why do they have exactly this many atoms? It is because there are many codes in the molecule of insulin, analogue codes and other coded features. In fact, there is a program-cybernetic algorithm in which it is „recorded" that the firs amino acid has to have 23 atoms, the second one 19, the third one 17, etc... The first amino acid has its own biochemistry, the second and the third one also. The conclusion here has to be that there is a concrete relationship between quantitative ratios in the process of transfer of genetic information and qualitative appearance, i.e. the characteristics of organisms

V. Prima Sequences

Number of atoms

V	**E**	**T**	**E**	**N**	**N**	**F**	**V**	**N**	**V**	**E**	**A**	**V**	**E**	**F**	**T**	**P**	**T**
1 9	**19**	**17**	**19**	**17**	**17**	**23**	**19**	**17**	**19**	**19**	**13**	**19**	**19**	**23**	**17**	**17**	**17**
3	**4**	**8**	**17**	**18**	**21**	**22**	**23**	**24**	**33**	**34**	**35**	**39**	**42**	**45**	**48**	**49**	**51**

V	E	T	E	N	N	F	V	N	V	E	A	V	E	F	T	P	T
19	19	17	19	17	17	23	19	17	19	19	13	19	19	23	17	17	17
54	55	59	68	69	72	73	74	75	84	85	86	90	93	96	99	100	102

V	E	T	E	N	N	F	V	N	V	E	A	V	E	F	T	P	T
19	19	17	19	17	17	23	19	17	19	19	13	19	19	23	17	17	17
207	208	212	221	222	225	226	227	228	237	238	239	243	246	249	252	253	255
V	E	T	E	N	N	F	V	N	V	E	A	V	E	F	T	P	T
19	19	17	19	17	17	23	19	17	19	19	13	19	19	23	17	17	17
258	259	263	272	273	276	277	278	279	288	289	290	294	297	300	303	304	306

Prima numbers 19, 19, 17,…, 17 = Prima sequences V,E,T,…T

In this fragments of Insulin there are 108 amino acids.

Decoding scheme

		Number of amino acids in Insulin		
		↓		
	Secondary sequence	306	Prima sequences	
	198		108	

	3660		1980	
	Number of atoms		Number of atoms	

(3660 x 198) – (1980 x 108) = DET 3660, 1980, 198, 108;

DET 2x2 = 3240;

V	E	T	E	N	N	V	E	T	E	N	N	V	E	T	E	N	N
F	V	N	V	E	A	F	V	N	V	E	A	F	V	N	V	E	A
V	E	F	T	P	T	V	E	F	T	P	T	V	E	F	T	P	T
V	E	T	E	N	N	V	E	T	E	N	N	V	E	T	E	N	N
F	V	N	V	E	A	F	V	N	V	E	A	F	V	N	V	E	A
V	E	F	T	P	T	V	E	F	T	P	T	V	E	F	T	P	T

Prima number of atoms

19	19	17	19	17	17	19	19	17	19	17	17	19	19	17	19	17	17
23	19	17	19	19	13	23	19	17	19	19	13	23	19	17	19	19	13
19	19	23	17	17	17	19	19	23	17	17	17	19	19	23	17	17	17
19	19	17	19	17	17	19	19	17	19	17	17	19	19	17	19	17	17
23	19	17	19	19	13	23	19	17	19	19	13	23	19	17	19	19	13
19	19	23	17	17	17	19	19	23	17	17	17	19	19	23	17	17	17

↓ **660** ↓ **660** ↓ **660**

Rank of amino acids

3	4	8	17	18	21	105	106	110	119	120	123	207	208	212	221	222	225
22	23	24	33	34	35	124	125	126	135	136	137	226	227	228	237	238	239
39	42	45	48	49	51	141	144	147	150	151	153	243	246	249	252	253	255
54	55	59	68	69	72	156	157	161	170	171	174	258	259	263	272	273	276
73	74	75	84	85	86	175	176	177	186	187	188	277	278	279	288	289	290
90	93	96	99	100	102	192	195	198	201	202	204	294	297	300	303	304	306

↓ **1950** ↓ **5622** ↓ **9294**

Correlation:

1950		5622		9294
			↙	
		11244		
	↙			
	5622		5622	

(1950 + 9294) = (5622 + 5622);

VI. Decoding scheme 2

We shall also seek the answers for the following questions: Does the matrix mechanism of biosynthesis of this protein function within the law of the general theory of information and theory of system, and what is the significance of it for understanding of the genetic language of insulin? What

is the essence of existence and functioning of this language? Is the genetic information characterized only by biochemical, or also by cyber-information principles? Etc..

Example 1

3	4	8	17	18	21	105	106	110	119	120	123	207	208	212	221	222	225
22	23	24	33	34	35	124	125	126	135	136	137	226	227	228	237	238	239
39	42	45	48	49	51	141	144	147	150	151	153	243	246	249	252	253	255
54	55	59	68	69	72	156	157	161	170	171	174	258	259	263	272	273	276
73	74	75	84	85	86	175	176	177	186	187	188	277	278	279	288	289	290
90	93	96	99	100	102	192	195	198	201	202	204	294	297	300	303	304	306
				↓					↓						↓		
				867					**2499**						**4131**		

Correlation

867		**2499**		**4131**
			↙	
		4998		
	2499		**2499**	

(867 + 4131) = (2499 + 2499);

Example 2

Dagonals

3	4	8	17	18	21	105	106	110	119	120	123	207	208	212	221	222	225
22	23	24	33	34	35	124	125	126	135	136	137	226	227	228	237	238	239
39	42	45	48	49	51	141	144	147	150	151	153	243	246	249	252	253	255
54	55	59	68	69	72	156	157	161	170	171	174	258	259	263	272	273	276
73	74	75	84	85	86	175	176	177	186	187	188	277	278	279	288	289	290
90	93	96	99	100	102	192	195	198	201	202	204	294	297	300	303	304	306

D1 = 326; D2=326; D3=938; D4=938; D5=1550; D6=1550;

Correlation:

326		938		1550
			↙	
		1876		
	↙		↘	
	938		938	

(326 + 1550) = (938 + 938);

Example 3

3	4	8	17	18	21
22	23	24	33	34	35
39	42	45	48	49	51
54	55	59	68	69	72
73	74	75	84	85	86
90	93	96	99	100	102

↓ **975**

105	106	110	119	120	123
124	125	126	135	136	137
141	144	147	150	151	153
156	157	161	170	171	174
175	176	177	186	187	188
192	195	198	201	202	204

↓ **2811**

207	208	212	221	222	225
226	227	228	237	238	239
243	246	249	252	253	255
258	259	263	272	273	276
277	278	279	288	289	290
294	297	300	303	304	306

↓ **4647**

Correlation:

975		**2811**		**4647**
			↙	
		5622		
	2811		**2811**	

(975 + 4647) = (2811 + 2811);

Example 4

				1	1	2
	3	4	7	8	1	
	2	2	2	3	3	3
2	3	4	3	4	5	
	3	4	4	4	4	5
9	2	5	8	9	1	
	5	5	5	6	6	7
4	5	9	8	9	2	
	7	7	7	8	8	8
3	4	5	4	5	6	
	9	9	9	9	1	1
0	3	6	9	00	02	

↓ **975**

	1	1	1	1	1	1
05	06	10	19	20	23	
	1	1	1	1	1	1
24	25	26	35	36	37	
	1	1	1	1	1	1
41	44	47	50	51	53	
	1	1	1	1	1	1
56	57	61	70	71	74	
	1	1	1	1	1	1
75	76	77	86	87	88	
	1	1	1	2	2	2
92	95	98	01	02	04	

↓ **2811**

	2	2	2	2	2	2
07	08	12	21	22	25	
	2	2	2	2	2	2
26	27	28	37	38	39	
	2	2	2	2	2	2
43	46	49	52	53	55	
	2	2	2	2	2	2
58	59	63	72	73	76	
	2	2	2	2	2	2
77	78	79	88	89	90	
	2	2	3	3	3	3
94	97	00	03	04	06	

↓ **4647**

Correlation:

975		**2811**		**4647**
			↙	
		5622		
			↘	
	2811		**2811**	

(975 + 4647) = (2811 + 2811);

Example 3 = Example 4

The result of the research that we have carried out clearly shows that there is a matrix code in insulin. It also shows that the coding system within the amino acidic language gives a full information, not only for the amino acid „record", but also for its structure, configuration and its various shapes. In the following text we shall discuss the issue of the existence of the insulin code, and also the issue of coding of individual structural levels in this protein.

VII. FRAGMENTS

3	4	8	17	18	21
22	23	24	33	34	35
39	42	45	48	49	51
54	55	59	68	69	72
73	74	75	84	85	86
90	93	96	99	100	102

895

105	106	110	119	120	123
124	125	126	135	136	137
141	144	147	150	151	153
156	157	161	170	171	174
175	176	177	186	187	188
192	195	198	201	202	204

3037

207	208	212	221	222	225
226	227	228	237	238	239
243	246	249	252	253	255
258	259	263	272	273	276
277	278	279	288	289	290
294	297	300	303	304	306

5179

3	4	8	17	18	21
22	23	24	33	34	35
39	42	45	48	49	51
54	55	59	68	69	72
73	74	75	84	85	86
90	93	96	99	100	102

1381

105	106	110	119	120	123
124	125	126	135	136	137
141	144	147	150	151	153
156	157	161	170	171	174
175	176	177	186	187	188
192	195	198	201	202	204

3523

207	208	212	221	222	225
226	227	228	237	238	239
243	246	249	252	253	255
258	259	263	272	273	276
277	278	279	288	289	290
294	297	300	303	304	306

5665

(1381-895) = (3523-3037) =(5665-5179)

		1	1	2	
3	4	8	7	8	1
2	2	2	3	3	3
2	3	4	3	4	5
3	4	4	4	4	5
9	2	5	8	9	1
5	5	5	6	6	7
4	5	9	8	9	2
7	7	7	8	8	8
3	4	5	4	5	6
9	9	9	9	1	1
0	3	6	9	00	02

7
86

1	1	1	1	1	1
05	06	10	19	20	23
1	1	1	1	1	1
24	25	26	35	36	37
1	1	1	1	1	1
41	44	47	50	51	53
1	1	1	1	1	1
56	57	61	70	71	74
1	1	1	1	1	1
75	76	77	86	87	88
1	1	1	2	2	2
92	95	98	01	02	04

2
928

2	2	2	2	2	2
07	08	12	21	22	25
2	2	2	2	2	2
26	27	28	37	38	39
2	2	2	2	2	2
43	46	49	52	53	55
2	2	2	2	2	2
58	59	63	72	73	76
2	2	2	2	2	2
77	78	79	88	89	90
2	2	3	3	3	3
94	97	00	03	04	06

5
070

(1381-786) = (3523-2928) = (5665-5070)

Example 5

		1	1	2	
3	4	87	8	1	
2	2	2	3	3	3
2	3	4	3	4	5

1	1	1	1	1	1
05	06	10	19	20	23
1	1	1	1	1	1
24	25	26	35	36	37

2	2	2	2	2	2
07	08	12	21	22	25
2	2	2	2	2	2
26	27	28	37	38	39

	3	4	4	4	4	5	1	1	1	1	1	1	2	2	2	2	2	2	
9	2	5	8	9	1	41	44	47	50	51	53	43	46	49	52	53	55		
	5	5	5	6	6	7	1	1	1	1	1	1	2	2	2	2	2	2	
4	5	9	8	9	2	56	57	61	70	71	74	58	59	63	72	73	76		
	7	7	7	8	8	8	1	1	1	1	1	1	2	2	2	2	2	2	
3	4	5	4	5	6	75	76	77	86	87	88	77	78	79	88	89	90		
	9	9	9	9	1	1	1	1	1	2	2	2	2	2	3	3	3	3	
0	3	6	9	00	02	92	95	98	01	02	04	94	97	00	03	04	06		
					↓					↓					↓				
					466					1282					2098				

Correlation:

4 66		**1 282**		**2 098**
			↙	
		2 564		
	1 282		**1 282**	

(466 + 2098) = (1282 + 1282);

Example 5

3	4	8	17	18	21	105	106	110	119	120	123	207	208	212	221	222	225
22	23	24	33	34	35	124	125	126	135	136	137	226	227	228	237	238	239
39	42	45	48	49	51	141	144	147	150	151	153	243	246	249	252	253	255
54	55	59	68	69	72	156	157	161	170	171	174	258	259	263	272	273	276
73	74	75	84	85	86	175	176	177	186	187	188	277	278	279	288	289	290
90	93	96	99	100	102	192	195	198	201	202	204	294	297	300	303	304	306
				↓					↓						↓		
				1190					3434						5678		

Correlation:

1 190		**3 434**		**5 678**
			↙	
		6 868		
	3 434		**3 434**	

(466 + 2098) = (1282 + 1282);

Example 6

3	4	8	17	18	21	105	106	110	119	120	123	207	208	212	221	222	225
22	23	24	33	34	35	124	125	126	135	136	137	226	227	228	237	238	239
39	42	45	48	49	51	141	144	147	150	151	153	243	246	249	252	253	255
54	55	59	68	69	72	156	157	161	170	171	174	258	259	263	272	273	276
73	74	75	84	85	86	175	176	177	186	187	188	277	278	279	288	289	290
90	93	96	99	100	102	192	195	198	201	202	204	294	297	300	303	304	306
				↓					↓						↓		
				967					2803						4639		

Correlation:

9 67		2 803		4 639
		5 606		
	2 803		2 803	

(466 + 2098) = (1282 + 1282);

3	4	8	17	18	21
22	23	24	33	34	35
39	42	45	48	49	51
54	55	59	68	69	72
73	74	75	84	85	86
90	93	96	99	100	102

869

105	106	110	119	120	123
124	125	126	135	136	137
141	144	147	150	151	153
156	157	161	170	171	174
175	176	177	186	187	188
192	195	198	201	202	204

2501

207	208	212	221	222	225
226	227	228	237	238	239
243	246	249	252	253	255
258	259	263	272	273	276
277	278	279	288	289	290
294	297	300	303	304	306

4133

(869 + 4133) : 2 = 2501;

3	4	8	17	18	21
22	23	24	33	34	35
39	42	45	48	49	51
54	55	59	68	69	72
73	74	75	84	85	86
90	93	96	99	100	102

863

105	106	110	119	120	123
124	125	126	135	136	137
141	144	147	150	151	153
156	157	161	170	171	174
175	176	177	186	187	188
192	195	198	201	202	204

2495

207	208	212	221	222	225
226	227	228	237	238	239
243	246	249	252	253	255
258	259	263	272	273	276
277	278	279	288	289	290
294	297	300	303	304	306

4127

(863 + 4127) : 2 = 2495;

3	4	8	17	18	21
22	23	24	33	34	35
39	42	45	48	49	51
54	55	59	68	69	72
73	74	75	84	85	86
90	93	96	99	100	102

866

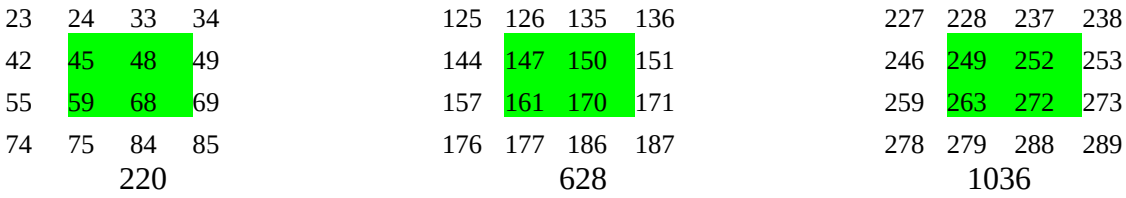
105	106	110	119	120	123
124	125	126	135	136	137
141	144	147	150	151	153
156	157	161	170	171	174
175	176	177	186	187	188
192	195	198	201	202	204

2498

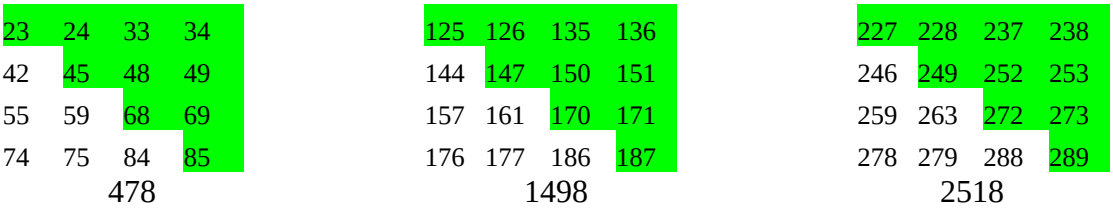
207	208	212	221	222	225
226	227	228	237	238	239
243	246	249	252	253	255
258	259	263	272	273	276
277	278	279	288	289	290
294	297	300	303	304	306

4130

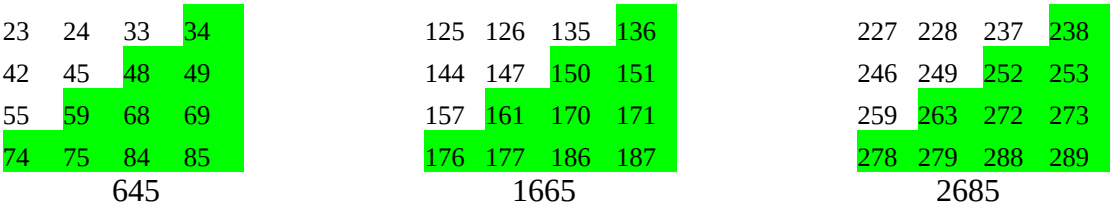
(866 + 4130) : 2 = 2498;



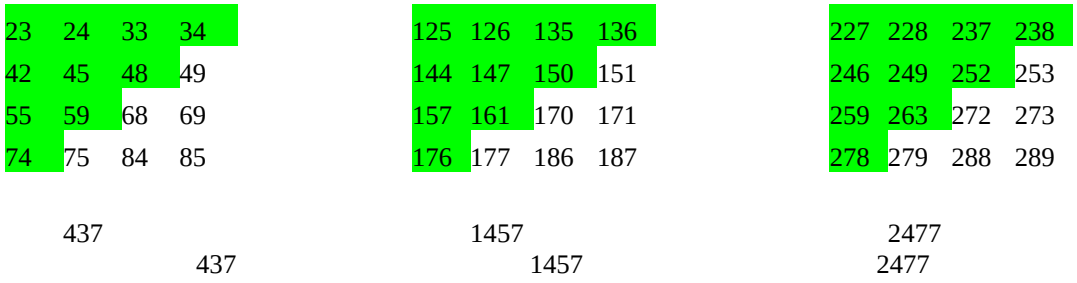
(220 + 1036) : 2 = 628;



(478 + 2518) : 2 = 1498;



(645-478) = (1665 – 1498) = (2685 – 2518)



(645-437) = (1665-1457) = (2685-2477)

The molecule of insulin we can understand as words built from letters, i.e. aminoacids. The meaning of words is determined by positioning of letters. Each of these words has its biochemical base. If this base is expressed by corresponding discrete numbers, we find out that the base has its own program, cybernetic and information etc.

characteristics. In fact, we will find out that the sequencing of the molecule is conditioned and determined not only by biochemical, but also by cybernetic and information principles For this reason, in this research we will deal more with quantitative, and less with qualitative characteristics of the genetic information and its biochemical foundation.

Groups 1

Amino acids-From 1 to 36

V	E	T	E	N	N
F	V	N	V	E	A
V	E	F	T	P	T
V	E	T	E	N	N
F	V	N	V	E	A
V	E	F	T	P	T

↓

Number of atoms

19	19	17	19	17	17
23	19	17	19	19	13
19	19	23	17	17	17
19	19	17	19	17	17
23	19	17	19	19	13
19	19	23	17	17	17

↓

Rank of amino acids

3	4	8	17	18	21
22	23	24	33	34	35
39	42	45	48	49	51
54	55	59	68	69	72
73	74	75	84	85	86
90	93	96	99	100	102

Groups 2

Amino acids-From 37 to 72

Decoding scheme:

Example 1

	A		B		
3	4	8	17	18	21
22	23	24	33	34	35
39	42	45	48	49	51
54	55	59	68	69	72
73	74	75	84	85	86
90	93	96	99	100	102
	C		D		

A = (3+4+8…, +45) = 210;
B = 306; C = 669; D = 765;
(A + D) = (B + C);

Example 2

	Rows						
1	3	4	8	17	18	21	71
2	22	23	24	33	34	35	171
3	39	42	45	48	49	51	274
4	54	55	59	68	69	72	377
5	73	74	75	84	85	86	477

6	90	93	96	99	100	102	580

(Row 1+ Row 6) = (Row 3+ Row 4) = 651;

Example 3

D1					D2
3	4	8	17	18	21
22	23	24	33	34	35
39	42	45	48	49	51
54	55	59	68	69	72
73	74	75	84	85	86
90	93	96	99	100	102

D1 = 326; D2 = 326;

etc.

From the previous examples we can see that this protein really has its quantitative characteristics. It can be concluded that there is a connection between quantitative characteristics in the process of transfer of genetic information and the qualitative appearance of given genetic processes.

Within the digital pictures in biochemistry, the physical and chemical parameters are in a strict compliance with programmatic, cybernetic and information principles. Each bar in the protein chain attracts only the corresponding aminoacid, and only the relevant aminoacid can be positioned at certain place in the chain. Each peptide chain can have the exact number of aminoacids necessary to meet the strictly determined mathematical conditioning. It can have as many atoms as necessary to meet the mathematical balance of the biochemical phenomenon at certain mathematical level, etc... the digital language of biochemistry has a countless number of codes and analogue codes, as well as other information content. These pictures enable us to realize the very essence of functioning of biochemical processes.

VIII. Discussion

The process of sequencing in bio-macromolecules is conditioned and determined not only through biochemical, but also through cybernetic and information principles. The digital pictures of biochemistry provide us with cybernetic and information interpretation of the scientific facts. Now we have the exact scientific proofs that there is a genetic language that can be described by the theory of systems and cybernetics, and which functions in accordance with certain principles.

It is rewarding to translate the biochemical language of amino acids into a digital language because it may be very useful for developing new methods for predicting protein sub-cellular localization, membrane protein type, protein structure secondary prediction or any other protein attributes.

This is because ever since the concept of Chou's pseudo amino acid composition was proposed 1,2, there have been many efforts to try to use various digital numbers to represent the 20 native amino acids in order to better reflect the sequence-order effects through the vehicle of pseudo amino acid composition. Some investigators used complexity measure factor [3], some used the values derived from the cellular automata [4-7], some used hydrophobic and/or hydrophilic values [8-16], some were through Fourier transform [17,18], and some used the physicochemical distance [19].

Now it is going to be possible to use a completely new strategy of research in genetics. However, observation of all these relations which are the outcome of the periodic law (actually, of the law of binary coding) is necessary, because it can be of great importance for decoding conformational forms and the stereo-chemical and digital structure of proteins.

IX. References

1) K.C. Chou, Gene Cloning & Expression Technologies, Chapter 4 (Weinrer, P.W.,And Lu, Q., Eds.), Eaton Publishing, Westborough, MA (2002), Pp. 57-70.

2) K.C. Chou, Prediction Of Protein Cellular Attributes Using Pseudo Amino Acid Composition PROTEINS: Structure, Function, And Genetics (Erratum: Ibid., 2001, Vol.44,60) 43 (2001) 246-255.

3) Xiao, S. Shao, Y. Ding, Z. Huang, Y. Huang, K. C. Chou, Using Complexitymeasure Factor To Predict Protein Subcellular Location, Amino Acids 28 (2005) 57-61.

4) Xiao, S. Shao, Y. Ding, Z. Huang, X. Chen, K. C. Chou, Using Cellular Automata Togenerate Image Representation For Biological Sequences, Amino Acids 28 (2005) 29-35

5) Xiao, S. Shao, Y. Ding, Z. Huang, X. Chen, K. C. Chou, An Application Of Genecomparative Image For Predicting The Effect On Replication Ratio By

HBV Virus Gene Missense Mutation, Journal Of Theoretical Biology 235 (2005) 555-565.

6) Xiao, S. H. Shao, Z. D. Huang, K. C. Chou, Using Pseudo Amino Acid Composition To Predict Protein Structural Classes: Approached With Complexity Measure Factor,
7) Journal Of Computational Chemistry 27 (2006) 478-482.

8) Xiao, S. H. Shao, Y. S. Ding, Z. D. Huang, K. C. Chou, Using Cellular Automata Images And Pseudo Amino Acid Composition To Predict Protein Sub-Cellular Location, Amino Acids 30 (2006) 49-54.

9) Chou, Using Amphiphilic Pseudo Amino Acid Composition To Predict Enzymesubfamily Classes, Bioinformatics 21 (2005) 10-19.

10) Chou, Y. D. Cai, Prediction Of Membrane Protein Types By Incorporating Amphipathic Effects, Journal Of Chemical Information And Modeling 45 (2005) 407-413.

11) Z. P. Feng, Prediction Of The Subcellular Location Of Prokaryotic Proteins Based On A New Representation Of The Amino Acid Composition, Biopolymers 58 (2001) 491-499.

12) Z. P. Feng, An Overview On Predicting The Subcellular Location Of A Protein, In Silico Biol 2 (2002) 291-303.

13) Wang, J. Yang, Z. J. Xu, K. C. Chou, SLLE For Predicting Membrane Protein Types, Journal Of Theoretical Biology 232 (2005) 7-15.

14) S. Q. Wang, J. Yang, K. C. Chou, Using Stacked Generalization To Predict Membraneprotein Types Based On Pseudo Amino Acid Composition, Journal Of Theoretical Biology, In Press (2006) Doi:10.1016/J.Jtbi.2006.1005.1006.

15) Wang, J. Yang, G. P. Liu, Z. J. Xu, K. C. Chou, Weighted-Support Vector Machines For Predicting Membrane Protein Types Based On Pseudo Amino Acid Composition, Protein Engineering, Design, And Selection 17 (2004) 509-516.

16) S. W. Zhang, Q. Pan, H. C. Zhang, Z. C. Shao, J. Y. Shi, Prediction Protein Homo- Oligomer Types By Pseudo Amino Acid Composition: Approached With An Improved Feature Extraction And Naive Bayes Feature Fusion, Amino Acids 30 (2006) 461-468.

17) Y. Gao, S. H. Shao, X. Xiao, Y. S. Ding, Y. S. Huang, Z. D. Huang, K. C. Chou, Using Pseudo Amino Acid Composition To Predict Protein Subcellular Location: Approached With Lyapunov Index, Bessel Function, And Chebyshev Filter, Amino Acids 28 (2005) 373- 376.

18) Y. Z. Guo, M. Li, M. Lu, Z. Wen, K. Wang, G. Li, J. Wu, Classifying G Protein- Coupled Receptors And Nuclear Receptors Based On Protein Power Spectrum From Fast Fourier Transform, Amino Acids 30 (2006) 397-402.

19) H. Liu, M. Wang, K. C. Chou, Low-Frequency Fourier Spectrum For Predicting Membrane Protein Types, Biochem Biophys Res Commun 336 (2005) 737-739.

20) K. C. Chou, Prediction Of Protein Subcellular Locations By Incorporating Quasi- Sequence-Order Effect, Biochemical & Biophysical Research Communications 278 (2000) 477-483.

21) K. C. Chou, A Novel Approach To Predicting Protein Structural Classes In A (20-1)-D Amino Acid Composition Space, Proteins: Structure, Function & Genetics 21 (1995) 319- 344.
22) C. Chou, C. T. Zhang, Predicting Protein Folding Types By Distance Functions That Make Allowances For Amino Acid Interactions, Journal Of Biological Chemistry 269 (1994) 22014-22020.

23) C. Chou, C. T. Zhang, Review: Prediction Of Protein Structural Classes, Critical Reviews In Biochemistry And Molecular Biology 30 (1995) 275-349.

24) C. Chou, D. W. Elrod, Protein Subcellular Location Prediction, Protein Engineering 12 (1999) 107-118.

25) K. C. Chou, Review: Prediction Of Protein Structural Classes And Subcellular Locations,Current Protein And Peptide Science 1 (2000) 171-208.

26) K. C. Chou, D. W. Elrod, Prediction Of Membrane Protein Types And Subcellular Locations, PROTEINS: Structure, Function, And Genetics 34 (1999) 137-153.

27) K. C. Chou, D. W. Elrod, Prediction Of Enzyme Family Classes, Journal Of Proteome Research 2 (2003) 183-190.

28) K. C. Chou, Y. D. Cai, Predicting Enzyme Family Class In A Hybridization Space, Protein Science 13 (2004) 2857-2863.
29) 28. K. C. Chou, D. W. Elrod, Bioinformatical Analysis Ofg-Protein-Coupled Receptors, Journal Of Proteome Research 1 (2002) 429-433.

30) K. C. Chou, Prediction Of G-Protein-Coupled Receptor Classes, Journal Of Proteome Research 4 (2005) 1413-1418.
31) K. C. Chou, Y. D. Cai, Prediction Of Protease Types In A Hybridization Space, Biochem. Biophys. Res. Comm. 339 (2006) 1015-1020.

32) K. C. Chou, Y. D. Cai, Predicting Protein-Protein Interactions From Sequences In A Hybridization Space, Journal Of Proteome Research 5 (2006) 316-322.

33) K. C. Chou, Y. D. Cai, W. Z. Zhong, Predicting Networking Couples For Metabolic Pathways Of Arabidopsis, EXCLI Journal 5 (2006) 55-65.

34) K. C. Chou, Y. D. Cai, Predicting Protein Quaternary Structure By Pseudo Amino Acid Composition, PROTEINS: Structure, Function, And Genetics 53 (2003) 282-289.

35) Kurić L. The Digital Language Of Amino Acids. Amino Acids (2007) 653-661.

36) Kurić L. The Atomic Genetic Code. J. Comput Sci Biol 2 (2009) 101-116.

37) Kurić L. Mesure Complexe Des Caracteristiques Dynamiques De Series Temporellesjournal De La Societe De Statistique De Paris"- Tome 127, No 2.1986

Global Journals Guidelines Handbook 2010

www.GlobalJournals.org

FELLOW OF INTERNATIONAL CONGRESS OF MEDICAL RESEARCH (FICMR)

- 'FICMR' title will be awarded to the person/institution after approval of Editor-in-Chief and Editorial Board. The title 'FICMR' can be added to name in the following manner:
  e.g. **Dr. Andrew Knoll, Ph.D., FICMR**
  Dr. Jhon Petter,M.D.,FICMR
- FICMR can submit two papers every year for publication without any charges. The paper will be sent to two peer reviewers. The paper will be published after the acceptance of peer reviewers and Editorial Board.
- Free unlimited Web-space will be allotted to 'FICMR 'along with subDomain to contribute and partake in our activities.
- A **professional email address** will be allotted free with unlimited email space.
- FICMR will be authorized to receive e-Journals -GJMR for the Lifetime.
- FICMR will be exempted from the registration fees of Seminar/Symposium/Conference/Workshop conducted internationally of GJMR (FREE of Charge).
- FICMR will be Honorable Guest of any gathering held.

ASSOCIATE OF INTERNATIONAL CONGRESS OF MEDICAL RESEARCH (AICMR)

- AICMR title will be awarded to the person/institution after approval of Editor-in-Chef and Editorial Board. The title 'AICMR' can be added to name in the following manner:
  eg. **Dr. Thomas Herry, Ph.D., AICMR**
- AICMR can submit one paper every year for publication without any charges. The paper will be sent to two peer reviewers. The paper will be published after the acceptance of peer reviewers and Editorial Board.
- Free 2GB Web-space will be allotted to 'FICMR' along with subDomain to contribute and participate in our activities.
- A professional email address will be allotted with free 1GB email space.
- AICMR will be authorized to receive e-Journal GJMR for lifetime.

ANNUAL MEMBER

- Annual Member will be authorized to receive e-Journal GJMR for one year(Journal Subscription for One year).
- The member will be allotted free 1 GB Web-space along with subDomain to contribute and participate in our activities.
- A professional email address will be allotted free 500 MB email space.
- .

PAPER PUBLICATION

- The members can publish paper once. The paper will be sent to two-peer reviewer. The paper will be published after the acceptance of peer reviewers and Editorial Board.

Process of submission of Research Paper

The Area or field of specialization may or may not be of any category as mentioned in 'Scope of Journal' menu of the GlobalJournals.org website. There are 37 Research Journal categorized with Six parental Journals GJCST, GJMR, GJRE, GJMBR, GJSFR, GJHSS. For Authors should prefer the mentioned categories. There are three widely used systems UDC, DDC and LCC. The details are available as 'Knowledge Abstract' at Home page. The major advantage of this coding is that, the research work will be exposed to and shared with all over the world as we are being abstracted and indexed worldwide.

The paper should be in proper format. The format can be downloaded from first page of 'Author Guideline' Menu. The Author is expected to follow the general rules as mentioned in this menu. The paper should be written in MS-Word Format (*.DOC,*.DOCX).

The Author can submit the paper either online or offline. The authors should prefer online submission.

Online Submission: There are three ways to submit your paper:

(A) (I) Register yourself using top right corner of Home page then Login from same place twice. If you are already registered, then login using your username and password.

(II) Choose corresponding Journal from "Research Journals" Menu.

(III) Click 'Submit Manuscript'. Fill required information and Upload the paper.

(B) If you are using Internet Explorer (Although Mozilla Firefox is preferred), then Direct Submission through Homepage is also available.

(C) If these two are not convenient, and then email the paper directly to dean@globaljournals.org as an attachment.

Offline Submission: Author can send the typed form of paper by Post. However, online submission should be preferred.

Preferred Author Guidelines

MANUSCRIPT STYLE INSTRUCTION (Must be strictly followed)

Page Size: 8.27" X 11'"

- Left Margin: 0.65
- Right Margin: 0.65
- Top Margin: 0.75
- Bottom Margin: 0.75
- Font type of all text should be Times New Roman.
- Paper Title should be of Font Size 24 with one Column section.
- Author Name in Font Size of 11 with one column as of Title.
- Abstract Font size of 9 Bold, "Abstract" word in Italic Bold.
- Main Text: Font size 10 with justified two columns section
- Two Column with Equal Column with of 3.38 and Gaping of .2
- First Character must be two lines Drop capped.
- Paragraph before Spacing of 1 pt and After of 0 pt.
- Line Spacing of 1 pt
- Large Images must be in One Column
- Numbering of First Main Headings (Heading 1) must be in Roman Letters, Capital Letter, and Font Size of 10.
- Numbering of Second Main Headings (Heading 2) must be in Alphabets, Italic, and Font Size of 10.

You can use your own standard format also.

Author Guidelines:

1. General,
2. Ethical Guidelines,
3. Submission of Manuscripts,
4. Manuscript's Category,
5. Structure and Format of Manuscript,
6. After Acceptance.

1. GENERAL

Before submitting your research paper, one is advised to go through the details as mentioned in following heads. It will be beneficial, while peer reviewer justify your paper for publication.

Scope

The Global Journals welcome the submission of original paper, review paper, survey article relevant to the all the streams of Philosophy and knowledge. The Global Journals is parental platform for Global Journal of Computer Science and Technology, Researches in Engineering, Medical Research, Science Frontier Research, Human Social Science, Management, and Business organization. The choice of specific field can be done otherwise as following in Abstracting and Indexing Page on this Website. As the all Global Journals are being abstracted and indexed (in process) by most of the reputed organizations. Topics of only narrow interest will not be accepted unless they have wider potential or consequences.

2. ETHICAL GUIDELINES

Authors should follow the ethical guidelines as mentioned below for publication of research paper and research activities.

Papers are accepted on strict understanding that the material in whole or in part has not been, nor is being, considered for publication elsewhere. If the paper once accepted by Global Journals and Editorial Board, will become the *copyright of the Global Journals.*

Authorship: The authors and coauthors should have active contribution to conception design, analysis and interpretation of findings. They should critically review the contents and drafting of the paper. All should approve the final version of the paper before submission

The Global Journals follows the definition of authorship set up by the Global Academy of Research and Development. According to the Global Academy of R&D authorship, criteria must be based on:

1) Substantial contributions to conception and acquisition of data, analysis and interpretation of the findings.

2) Drafting the paper and revising it critically regarding important academic content.

3) Final approval of the version of the paper to be published.

All authors should have been credited according to their appropriate contribution in research activity and preparing paper. Contributors who do not match the criteria as authors may be mentioned under Acknowledgement.

Acknowledgements: Contributors to the research other than authors credited should be mentioned under acknowledgement. The specifications of the source of funding for the research if appropriate can be included. Suppliers of resources may be mentioned along with address.

Appeal of Decision: The Editorial Board's decision on publication of the paper is final and cannot be appealed elsewhere.

Permissions: It is the author's responsibility to have prior permission if all or parts of earlier published illustrations are used in this paper.

Please mention proper reference and appropriate acknowledgements wherever expected.

If all or parts of previously published illustrations are used, permission must be taken from the copyright holder concerned. It is the author's responsibility to take these in writing.

Approval for reproduction/modification of any information (including figures and tables) published elsewhere must be obtained by the authors/copyright holders before submission of the manuscript. Contributors (Authors) are responsible for any copyright fee involved.

3. SUBMISSION OF MANUSCRIPTS

Manuscripts should be uploaded via this online submission page. The online submission is most efficient method for submission of papers, as it enables rapid distribution of manuscripts and consequently speeds up the review procedure. It also enables authors to know the status of their own manuscripts by emailing us. Complete instructions for submitting a paper is available below.

Manuscript submission is a systematic procedure and little preparation is required beyond having all parts of your manuscript in a given format and a computer with an Internet connection and a Web browser. Full help and instructions are provided on-screen. As an author, you will be prompted for login and manuscript details as Field of Paper and then to upload your manuscript file(s) according to the instructions.

To avoid postal delays, all transaction is preferred by e-mail. A finished manuscript submission is confirmed by e-mail immediately and your paper enters the editorial process with no postal delays. When a conclusion is made about the publication of your paper by our Editorial Board, revisions can be submitted online with the same procedure, with an occasion to view and respond to all comments.

Complete support for both authors and co-author is provided.

4. MANUSCRIPT'S CATEGORY

Based on potential and nature, the manuscript can be categorized under the following heads: Original research paper: Such papers are reports of high-level significant original research work.

Review papers: These are concise, significant but helpful and decisive topics for young researchers.

Research articles: These are handled with small investigation and applications

Research letters: The letters are small and concise comments on previously published matters.

5. STRUCTURE AND FORMAT OF MANUSCRIPT

The recommended size of original research paper is less than seven thousand words, review papers fewer than seven thousands words also. Preparation of research paper or how to write research paper, are major hurdle, while writing manuscript. The research articles and research letters should be fewer than three thousand words, the structure original research paper; sometime review paper should be as follows:

Papers*:* These are reports of significant research (typically less than 7000 words equivalent, including tables, figures, references), and comprise:

(a)*Title* should be relevant and commensurate with the theme of the paper.

(b) A brief Summary, *"Abstract"* (less than 150 words) containing the major results and conclusions.

(c) Up to *ten keywords*, that precisely identifies the paper's subject, purpose, and focus.

(d) An *Introduction*, giving necessary background excluding subheadings; objectives must be clearly declared.

(e) Resources and techniques with sufficient complete experimental details (wherever possible by reference) to permit repetition; sources of information must be given and numerical methods must be specified by reference, unless non-standard.

(f) Results should be presented concisely, by well-designed tables and/or figures; the same data may not be used in both; suitable statistical data should be given. All data must be obtained with attention to numerical detail in the planning stage. As reproduced design has been recognized to be important to experiments for a considerable time, the Editor has decided that any paper that appears not to have adequate numerical treatments of the data will be returned un-refereed;

(g) Discussion should cover the implications and consequences, not just recapitulating the results; *conclusions* should be summarizing.

(h) Brief Acknowledgements.

(i) References in the proper form.

Authors should very cautiously consider the preparation of papers to ensure that they communicate efficiently. Papers are much more likely to be accepted, if they are cautiously designed and laid out, contain few or no errors, are summarizing, and be conventional to the approach and instructions. They will in addition, be published with much less delays than those that require much technical and editorial correction.

The Editorial Board reserves the right to make literary corrections and to make suggestions to improve briefness.

It is vital, that authors take care in submitting a manuscript that is written in simple language and adheres to published guidelines.

Format

Language: The language of publication is UK English. Authors, for whom English is a second language, must have their manuscript efficiently edited by an English-speaking person before submission to make sure that, the English is of high excellence. It is preferable, that manuscripts should be professionally edited.

Standard Usage, Abbreviations, and Units: Spelling and hyphenation should be conventional to The Concise Oxford English Dictionary. Statistics and measurements should at all times be given in figures, e.g. 16 min, except for when the number begins a sentence. When the number does not refer to a unit of measurement it should be spelt in full unless, it is 160 or greater.

Abbreviations supposed to be used carefully. The abbreviated name or expression is supposed to be cited in full at first usage, followed by the conventional abbreviation in parentheses.

Metric SI units are supposed to generally be used excluding where they conflict with current practice or are confusing. For illustration, 1.4 l rather than 1.4 × 10-3 m3, or 4 mm somewhat than 4 × 10-3 m. Chemical formula and solutions must identify the form used, e.g. anhydrous or hydrated, and the concentration must be in clearly defined units. Common species names should be followed by underlines at the first mention. For following use the generic name should be constricted to a single letter, if it is clear.

Structure

All manuscripts submitted to Global Journals, ought to include:

Title: The title page must carry an instructive title that reflects the content, a running title (less than 45 characters together with spaces), names of the authors and co-authors, and the place(s) wherever the work was carried out. The full postal address in addition with the e-mail address of related author must be given. Up to eleven keywords or very brief phrases have to be given to help data retrieval, mining and indexing.

Abstract, used in Original Papers and Reviews:

Optimizing Abstract for Search Engines

Many researchers searching for information online will use search engines such as Google, Yahoo or similar. By optimizing your paper for search engines, you will amplify the chance of someone finding it. This in turn will make it more likely to be viewed and/or cited in a further work. Global Journals have compiled these guidelines to facilitate you to maximize the web-friendliness of the most public part of your paper.

Keywords

A major linchpin in research work for the writing research paper is the keyword search, which one will employ to find both library and Internet resources.

One must be persistent and creative in using keywords. An effective keyword search requires a strategy and planning a list of possible keywords and phrases to try.

Search engines for most searches, use Boolean searching, which is somewhat different from Internet searches. The Boolean search uses "operators," words (and, or, not, and near) that enable you to expand or narrow your affords. Tips for research paper while preparing research paper are very helpful guideline of research paper.

Choice of key words is first tool of tips to write research paper. Research paper writing is an art.A few tips for deciding as strategically as possible about keyword search:

- One should start brainstorming lists of possible keywords before even begin searching. Think about the most important concepts related to research work. Ask, "What words would a source have to include to be truly valuable in research paper?" Then consider synonyms for the important words.
- It may take the discovery of only one relevant paper to let steer in the right keyword direction because in most databases, the keywords under which a research paper is abstracted are listed with the paper.
- One should avoid outdated words.

Keywords are the key that opens a door to research work sources. Keyword searching is an art in which researcher's skills are bound to improve with experience and time.

Numerical Methods: Numerical methods used should be clear and, where appropriate, supported by references.

Acknowledgements: Please make these as concise as possible.

References

References follow the *Harvard scheme* of referencing. References in the text should cite the authors' names followed by the time of their publication, unless there are three or more authors when simply the first author's name is quoted followed by et al. unpublished work has to only be cited where necessary, and only in the text. Copies of references in press in other journals have to be supplied with submitted typescripts. It is necessary that all citations and references be carefully checked before submission, as mistakes or omissions will cause delays.

References to information on the World Wide Web can be given, but only if the information is available without charge to readers on an official site. Wikipedia and Similar websites are not allowed where anyone can change the information. Authors will be asked to make available electronic copies of the cited information for inclusion on the Global Journals homepage at the judgment of the Editorial Board.

The Editorial Board and Global Journals recommend that, citation of online-published papers and other material should be done via a DOI (digital object identifier). If an author cites anything, which does not have a DOI, they run the risk of the cited material not being noticeable.

The Editorial Board and Global Journals recommend the use of a tool such as Reference Manager for reference management and formatting.

Tables, Figures and Figure Legends

Tables: Tables should be few in number, cautiously designed, uncrowned, and include only essential data. Each must have an Arabic number, e.g. Table 4, a self-explanatory caption and be on a separate sheet. Vertical lines should not be used.

Figures: Figures are supposed to be submitted as separate files. Always take in a citation in the text for each figure using Arabic numbers, e.g. Fig. 4. Artwork must be submitted online in electronic form by e-mailing them.

Preparation of Electronic Figures for Publication

Even though low quality images are sufficient for review purposes, print publication requires high quality images to prevent the final product being blurred or fuzzy. Submit (or e-mail) EPS (line art) or TIFF (halftone/photographs) files only. MS PowerPoint and Word Graphics are unsuitable for printed pictures. Do not use pixel-oriented software. Scans (TIFF only) should have a resolution of at least 350 dpi (halftone) or 700 to 1100 dpi (line drawings) in relation to the imitation size. Please give the data for figures in black and white or submit a Color Work Agreement Form. EPS files must be saved with fonts embedded (and with a TIFF preview, if possible).

For scanned images, the scanning resolution (at final image size) ought to be as follows to ensure good reproduction: line art: >650 dpi; halftones (including gel photographs) : >350 dpi; figures containing both halftone and line images: >650 dpi.

Color Charges: It is the rule of the Global Journals for authors to pay the full cost for the reproduction of their color artwork. Hence, please note that, if there is color artwork in your manuscript when it is accepted for publication, we would require you to complete and return a color work agreement form before your paper can be published.

Figure Legends: Self-explanatory legends of all figures should be incorporated separately under the heading 'Legends to Figures'. In the full-text online edition of the journal, figure legends may possibly be truncated in abbreviated links to the full screen version. Therefore, the first 100 characters of any legend should notify the reader, about the key aspects of the figure.

6. AFTER ACCEPTANCE

Upon approval of a paper for publication, the manuscript will be forwarded to the dean, who is responsible for the publication of the Global Journals.

6.1 Proof Corrections

The corresponding author will receive an e-mail alert containing a link to a website or will be attached. A working e-mail address must therefore be provided for the related author.

Acrobat Reader will be required in order to read this file. This software can be downloaded
(Free of charge) from the following website:

www.adobe.com/products/acrobat/readstep2.html. This will facilitate the file to be opened, read on screen, and printed out in order for any corrections to be added. Further instructions will be sent with the proof.

Proofs must be returned to the dean at dean@globaljournals.org within three days of receipt.

As changes to proofs are costly, we inquire that you only correct typesetting errors. All illustrations are retained by the publisher. Please note that the authors are responsible for all statements made in their work, including changes made by the copy editor.

6.2 Early View of Global Journals (Publication Prior to Print)

The Global Journals are enclosed by our publishing's Early View service. Early View articles are complete full-text articles sent in advance of their publication. Early View articles are absolute and final. They have been completely reviewed, revised and edited for publication, and the authors' final corrections have been incorporated. Because they are in final form, no changes can be made after sending them. The nature of Early View articles means that they do not yet have volume, issue or page numbers, so Early View articles cannot be cited in the conventional way.

6.3 Author Services

Online production tracking is available for your article through Author Services. Author Services enables authors to track their article - once it has been accepted - through the production process to publication online and in print. Authors can check the status of their articles online and choose to receive automated e-mails at key stages of production. The authors will receive an e-mail with a unique link that enables them to register and have their article automatically added to the system. Please ensure that a complete e-mail address is provided when submitting the manuscript.

6.4 Author Material Archive Policy

Please note that if not specifically requested, publisher will dispose off hardcopy & electronic information submitted, after the two months of publication. If you require the return of any information submitted, please inform the Editorial Board or dean as soon as possible.

6.5 Offprint and Extra Copies

A PDF offprint of the online-published article will be provided free of charge to the related author, and may be distributed according to the Publisher's terms and conditions. Additional paper offprint may be ordered by emailing us at: editor@globaljournals.org .

INFORMAL TIPS FOR WRITING A MEDICAL RESEARCH PAPER TO INCREASE READABILITY AND CITATION

Before start writing a good quality Medical Research Paper, let us first understand what is Medical Research Paper? So, Medical Research Paper is the paper which is written by professionals or scientists who are associated to medical, medicine, pharmacy, chemistry, and health, Or doing research study in these areas. If you are novel to this field then you can consult about this field from your supervisor or guide.

Techniques for writing a good quality Medical Research Paper:

1. Choosing the topic- In most cases, the topic is searched by the interest of author but it can be also suggested by the guides. You can have several topics and then you can judge that in which topic or subject you are finding yourself most comfortable. This can be done by asking several questions to yourself, like Will I be able to carry our search in this area? Will I find all necessary recourses to accomplish the search? Will I be able to find all information in this field area? If the answer of these types of questions will be "Yes" then you can choose that topic. In most of the cases, you may have to conduct the surveys and have to visit several places because this field is related to medical. Also, you may have to do a lot of work to find all rise and falls regarding the various data of that subject. Sometimes, detailed information plays a vital role, instead of short information.

2. Evaluators are human: First thing to remember that evaluators are also human being. They are not only meant for rejecting a paper. They are here to evaluate your paper. So, present your Best.

3. Think Like Evaluators: If you are in a confusion or getting demotivated that your paper will be accepted by evaluators or not, then think and try to evaluate your paper like an Evaluator. Try to understand that what an evaluator wants in your research paper and automatically you will have your answer.

4. Make blueprints of paper: The outline is the plan or framework that will help you to arrange your thoughts. It will make your paper logical. But remember that all points of your outline must be related to the topic you have chosen.

5. Ask your Guides: If you are having any difficulty in your research, then do not hesitate to share your difficulty to your guide (if you have any). They will surely help you out and resolve your doubts. If you can't clarify what exactly you require for your work then ask the

supervisor to help you with the alternative. He might also provide you the list of essential readings.

6. Use of computer is recommended: At a first glance, this point looks obvious but it is first recommendation that to write a quality research paper of any area, first draft your paper in Microsoft Word. By using MS Word, you can easily catch your grammatical mistakes and spelling errors.

7. Use right software: Always use good quality software packages. If you are not capable to judge good software then you can lose quality of your paper unknowingly. There are various software programs available to help you, which you can get through Internet.

8. Use the Internet for help: An excellent start for your paper can be by using the Google. It is an excellent search engine, where you can have your doubts resolved. You may also read some answers for the frequent question how to write my research paper or find model research paper. From the internet library you can download books. If you have all required books make important reading selecting and analyzing the specified information. Then put together research paper sketch out.

9. Use and get big pictures: Always use encyclopedias, Wikipedia to get pictures so that you can go into the depth.

10. Bookmarks are useful: When you read any book or magazine, you generally use bookmarks, right! It is a good habit, which helps to not to lose your continuity. You should always use bookmarks while searching on Internet also, which will make your search easier.

11. Revise what you wrote: When you write anything, always read it, summarize it and then finalize it.

12. Make all efforts: Make all efforts to mention what you are going to write in your paper. That means always have a good start. Try to mention everything in introduction, that what is the need of a particular research paper. Polish your work by good skill of writing and always give an evaluator, what he wants.

13. Have backups: When you are going to do any important thing like making research paper, you should always have backup copies of it either in your computer or in paper. This will help you to not to lose any of your important.

14. Produce good diagrams of your own: Always try to include good charts or diagrams in your paper to improve quality. Using several and unnecessary diagrams will degrade the quality of your paper by creating "hotchpotch." So always, try to make and include those diagrams, which are made by your own to improve readability and understandability of your paper.

15. Use of direct quotes: When you do research relevant to literature, history or current affairs then use of quotes become essential but if study is relevant to science then use of quotes is not preferable.

16. Use proper verb tense: Use proper verb tenses in your paper. Use past tense, to present those events that happened. Use present tense to indicate events that are going on. Use future tense to indicate future happening events. Use of improper and wrong tenses will confuse the evaluator. Avoid the sentences that are incomplete.

17. Never use online paper: If you are getting any paper on Internet, then never use it as your research paper because it might be possible that evaluator has already seen it or maybe it is outdated version.

18. Pick a good study spot: To do your research studies always try to pick a spot, which is quiet. Every spot is not for studies. Spot that suits you choose it and proceed further.

19. Know what you know: Always try to know, what you know by making objectives. Else, you will be confused and cannot achieve your target.

20. Use good quality grammar: Always use a good quality grammar and use words that will throw positive impact on evaluator. Use of good quality grammar does not mean to use tough words, that for each word the evaluator has to go through dictionary. Do not start sentence with a conjunction. Do not fragment sentences. Eliminate one-word sentences. Ignore passive voice. Do not ever use a big word when a diminutive one would suffice. Verbs have to be in agreement with their subjects. Prepositions are not expressions to finish sentences with. It is incorrect to ever divide an infinitive. Avoid clichés like the disease. Also, always shun irritating alliteration. Use language that is simple and straight forward. put together a neat summary.

21. Arrangement of information: Each section of the main body should start with an opening sentence and there should be a changeover at the end of the section. Give only valid and powerful arguments to your topic. You may also maintain your arguments with records.

22. Never start in last minute: Always start at right time and give enough time to research work. Leaving everything to the last minute

will degrade your paper and spoil your work.

23. Multitasking in research is not good: Doing several things at the same time proves bad habit in case of research activity. Research is an area, where everything has a particular time slot. Divide your research work in parts and do particular part in particular time slot.

24. Never copy others' work: Never copy others' work and give it your name because if evaluator has seen it anywhere you will be in trouble.

25. Take proper rest and food: No matter how many hours you spend for your research activity, if you are not taking care of your health then all your efforts will be in vain. For a quality research, study is must, and this can be done by taking proper rest and food.

26. Go for seminars: Attend seminars if the topic is relevant to your research area. Utilize all your resources.

27. Refresh your mind after intervals: Try to give rest to your mind by listening to soft music or by sleeping in intervals. This will also improve your memory.

28. Make colleagues: Always try to make colleagues. No matter how sharper or intelligent you are, if you make colleagues you can have several ideas, which will be helpful for your research.

29. Think technically: Always think technically. If anything happens, then search its reasons, its benefits, and demerits.

30. Think and then print: When you will go to print your paper, notice that tables are not be split, headings are not detached from their descriptions, and page sequence is maintained.

31. Adding unnecessary information: Do not add unnecessary information, like, I have used MS Excel to draw graph. Do not add irrelevant and inappropriate material. These all will create superfluous. Foreign terminology and phrases are not apropos. One should NEVER take a broad view. Analogy in script is like feathers on a snake. Not at all use a large word when a very small one would be sufficient. Use words properly, regardless of how others use them. Remove quotations. Puns are for kids, not grunt readers. Amplification is a billion times of inferior quality than sarcasm.

32. Never oversimplify everything: To add material in your research paper, never go for oversimplification. This will definitely irritate the evaluator. Be more or less specific. Also too, by no means, ever use rhythmic redundancies. Contractions aren't essential and shouldn't be there used. Comparisons are as terrible as clichés. Give up ampersands and abbreviations, and so on. Remove commas, that are, not necessary. Parenthetical words however should be together with this in commas. Understatement is all the time the complete best way to put onward earth-shaking thoughts. Give a detailed literary review.

33. Report concluded results: Use concluded results. From raw data, filter the results and then conclude your studies based on measurements and observations taken. Significant figures and appropriate number of decimal places should be used. Parenthetical remarks are prohibitive. Proofread carefully at final stage. In the end give outline to your arguments. Spot out perspectives of further study of this subject. Justify your conclusion by at the bottom of them with sufficient justifications and examples.

34. After conclusion: Once you have concluded your research, the next most important step is to present your findings. Presentation is extremely important as it is the definite medium though which your research is going to be in print to the rest of the crowd. Care should be taken to categorize your thoughts well and present them in a logical and neat manner. A good quality research paper format is essential because it serves to highlight your research paper and bring to light all necessary aspects in your research.

Informal Guidelines of Research Paper Writing

Key points to remember:

- Submit all work in its final form.
- Write your paper in the form, which is presented in the guidelines using the template.
- Please note the criterion for grading the final paper by peer-reviewers.

Final Points:

A purpose of organizing a research paper is to let people to interpret your effort selectively. The journal requires the following sections,

submitted in the order listed, each section to start on a new page.

The introduction will be compiled from reference matter and will reflect the design processes or outline of basis that direct you to make study. As you will carry out the process of study, the method and process section will be constructed as like that. The result segment will show related statistics in nearly sequential order and will direct the reviewers next to the similar intellectual paths throughout the data that you took to carry out your study. The discussion section will provide understanding of the data and projections as to the implication of the results. The use of good quality references all through the paper will give the effort trustworthiness by representing an alertness of prior workings.

Writing a research paper is not an easy job no matter how trouble-free the actual research or concept. Practice, excellent preparation, and controlled record keeping are the only means to make straightforward the progression.

General style:

Specific editorial column necessities for compliance of a manuscript will always take over from directions in these general guidelines.

To make a paper clear

· Adhere to recommended page limits

Mistakes to evade

- Insertion a title at the foot of a page with the subsequent text on the next page
- Separating a table/chart or figure - impound each figure/table to a single page
- Submitting a manuscript with pages out of sequence

In every sections of your document

· Use standard writing style including articles ("a", "the," etc.)

· Keep on paying attention on the research topic of the paper

· Use paragraphs to split each significant point (excluding for the abstract)

· Align the primary line of each section

· Present your points in sound order

· Use present tense to report well accepted

· Use past tense to describe specific results

· Shun familiar wording, don't address the reviewer directly, and don't use slang, slang language, or superlatives

· Shun use of extra pictures - include only those figures essential to presenting results

Title Page:

Choose a revealing title. It should be short. It should not have non-standard acronyms or abbreviations. It should not exceed two printed lines. It should include the name(s) and address (es) of all authors.

Abstract:

The summary should be two hundred words or less. It should briefly and clearly explain the key findings reported in the manuscript--must have precise statistics. It should not have abnormal acronyms or abbreviations. It should be logical in itself. Shun citing references at this point.

An abstract is a brief distinct paragraph summary of finished work or work in development. In a minute or less a reviewer can be taught the foundation behind the study, common approach to the problem, relevant results, and significant conclusions or new questions.

Write your summary when your paper is completed because how can you write the summary of anything which is not yet written? Wealth of terminology is very essential in abstract. Yet, use comprehensive sentences and do not let go readability for briefness. You can maintain it succinct by phrasing sentences so that they provide more than lone rationale. The author can at this moment go straight to shortening the outcome. Sum up the study, with the subsequent elements in any summary. Try to maintain the initial two items to no more than one ruling each.

- Reason of the study - theory, overall issue, purpose
- Fundamental goal
- To the point depiction of the research
- Consequences, including definite statistics - if the consequences are quantitative in nature, account quantitative data; results of any numerical analysis should be reported
- Significant conclusions or questions that track from the research(es)

Approach:

- Single section, and succinct
- As a outline of job done, it is always written in past tense
- A conceptual should situate on its own, and not submit to any other part of the paper such as a form or table
- Center on shortening results - bound background information to a verdict or two, if completely necessary
- What you account in an conceptual must be regular with what you reported in the manuscript
- Exact spelling, clearness of sentences and phrases, and appropriate reporting of quantities (proper units, important statistics) are just as significant in an abstract as they are anywhere else

Introduction:

The **Introduction** should "introduce" the manuscript. The reviewer should be presented with sufficient background information to be capable to comprehend and calculate the purpose of your study without having to submit to other works. The basis for the study should be offered. Give most important references but shun difficult to make a comprehensive appraisal of the topic. In the introduction, describe the problem visibly. If the problem is not acknowledged in a logical, reasonable way, the reviewer will have no attention in your result. Speak in common terms about techniques used to explain the problem, if needed, but do not present any particulars about the protocols here. Following approach can create a valuable beginning:

- Explain the value (significance) of the study
- Shield the model - why did you employ this particular system or method? What is its compensation? You strength remark on its appropriateness from a abstract point of vision as well as point out sensible reasons for using it.
- Present a justification. Status your particular theory (es) or aim(s), and describe the logic that led you to choose them.
- Very for a short time explain the tentative propose and how it skilled the declared objectives.

Approach:

- Use past tense except for when referring to recognized facts. After all, the manuscript will be submitted after the entire job is done.
- Sort out your thoughts; manufacture one key point with every section. If you make the four points listed above, you will need a least of four paragraphs.
- Present surroundings information only as desirable in order hold up a situation. The reviewer does not desire to read the whole thing you know about a topic.
- Shape the theory/purpose specifically - do not take a broad view.
- As always, give awareness to spelling, simplicity and correctness of sentences and phrases.

Procedures (Methods and Materials):

This part is supposed to be the easiest to carve if you have good skills. A sound written Procedures segment allows a capable scientist to replacement your results. Present precise information about your supplies. The suppliers and clarity of reagents can be helpful bits of information. Present methods in sequential order but linked methodologies can be grouped as a segment. Be concise when relating the protocols. Attempt for the least amount of information that would permit another capable scientist to spare your outcome but be cautious that vital information is integrated. The use of subheadings is suggested and ought to be synchronized with the results section. When a technique is used that has been well described in another object, mention the specific item describing a way but draw the basic principle while stating the situation. The purpose is to text all particular resources and broad procedures, so that another person may use some or all of the methods in one more study or referee the scientific value of your work. It is not to be a step by step report of the whole thing you did, nor is a methods section a set of orders.

Materials:

- Explain materials individually only if the study is so complex that it saves liberty this way.
- Embrace particular materials, and any tools or provisions that are not frequently found in laboratories.
- Do not take in frequently found.
- If use of a definite type of tools.
- Materials may be reported in a part section or else they may be recognized along with your measures.

Methods:

- Report the method (not particulars of each process that engaged the same methodology)
- Describe the method entirely
- To be succinct, present methods under headings dedicated to specific dealings or groups of measures
- Simplify - details how procedures were completed not how they were exclusively performed on a particular day.
- If well known procedures were used, account the procedure by name, possibly with reference, and that's all.

Approach:

- It is embarrassed or not possible to use vigorous voice when documenting methods with no using first person, which would focus the reviewer's interest on the researcher rather than the job. As a result when script up the methods most authors use third person passive voice.
- Use standard style in this and in every other part of the paper - avoid familiar lists, and use full sentences.

What to keep away from

- Resources and methods are not a set of information.
- Skip all descriptive information and surroundings - save it for the argument.
- Leave out information that is immaterial to a third party.

Results:

The principle of a results segment is to present and demonstrate your conclusion. Create this part a entirely objective details of the outcome, and save all understanding for the discussion.

The page length of this segment is set by the sum and types of data to be reported. Carry on to be to the point, by means of statistics and tables, if suitable, to present consequences most efficiently.

You must obviously differentiate material that would usually be incorporated in a study editorial from any unprocessed data or additional appendix matter that would not be available. In fact, such matter should not be submitted at all except requested by the instructor.

Content

- Sum up your conclusion in text and demonstrate them, if suitable, with figures and tables.

- In manuscript, explain each of your consequences, point the reader to remarks that are most appropriate.
- Present a background, such as by describing the question that was addressed by creation an exacting study.
- Explain results of control experiments and comprise remarks that are not accessible in a prescribed figure or table, if appropriate.
- Examine your data, then prepare the analyzed (transformed) data in the form of a figure (graph), table, or in manuscript form.

What to stay away from

- Do not discuss or infer your outcome, report surroundings information, or try to explain anything.
- Not at all take in raw data or intermediate calculations in a research manuscript.
- Do not present the similar data more than once.
- Manuscript should complement any figures or tables, not duplicate the identical information.
- Never confuse figures with tables - there is a difference.

Approach

- As forever, use past tense when you submit to your results, and put the whole thing in a reasonable order.
- Put figures and tables, appropriately numbered, in order at the end of the report
- If you desire, you may place your figures and tables properly within the text of your results part.

Figures and tables

- If you put figures and tables at the end of the details, make certain that they are visibly distinguished from any attach appendix materials, such as raw facts
- Despite of position, each figure must be numbered one after the other and complete with subtitle
- In spite of position, each table must be titled, numbered one after the other and complete with heading
- All figure and table must be adequately complete that it could situate on its own, divide from text

Discussion:

The Discussion is expected the trickiest segment to write and describe. A lot of papers submitted for journal are discarded based on problems with the Discussion. There is no head of state for how long a argument should be. Position your understanding of the outcome visibly to lead the reviewer through your conclusions, and then finish the paper with a summing up of the implication of the study. The purpose here is to offer an understanding of your results and hold up for all of your conclusions, using facts from your research and generally accepted information, if suitable. The implication of result should be visibly described. Infer your data in the conversation in suitable depth. This means that when you clarify an observable fact you must explain mechanisms that may account for the observation. If your results vary from your prospect, make clear why that may have happened. If your results agree, then explain the theory that the proof supported. It is never suitable to just state that the data approved with prospect, and let it drop at that.

- Make a decision if each premise is supported, discarded, or if you cannot make a conclusion with assurance. Do not just dismiss a study or part of a study as "uncertain."
- Research papers are not acknowledged if the work is imperfect. Draw what conclusions you can based upon the results that you have, and take care of the study as a finished work
- You may propose future guidelines, such as how the experiment might be personalized to accomplish a new idea.
- Give details all of your remarks as much as possible, focus on mechanisms.
- Make a decision if the tentative design sufficiently addressed the theory, and whether or not it was correctly restricted.
- Try to present substitute explanations if sensible alternatives be present.
- One research will not counter an overall question, so maintain the large picture in mind, where do you go next? The best studies unlock new avenues of study. What questions remain?
- Recommendations for detailed papers will offer supplementary suggestions.

Approach:

- When you refer to information, differentiate data generated by your own studies from available information
- Submit to work done by specific persons (including you) in past tense.
- Submit to generally acknowledged facts and main beliefs in present tense.

Administration Rules Listed Before Submitting Your Research Paper to Global Journals

Please carefully note down following rules and regulation before submitting your Research Paper to Global Journals:

Segment Draft and Final Research Paper: You have to strictly follow the template of research paper. If it is not done your paper may get rejected.

- The **major constraint** is that you must independently make all content, tables, graphs, and facts that are offered in the paper. You must write each part of the paper wholly on your own. The Peer-reviewers need to identify your own perceptive of the concepts in your own terms. NEVER extract straight from any foundation, and never rephrase someone else's analysis.
- Do not give permission to anyone else to "PROOFREAD" your manuscript.

Written Material: You may discuss with your guides and key sources.

- Do not copy or imitate anyone else paper. (Various Methods to avoid Plagiarism is applied by us on every paper, if found guilty, you will be blacklisted by all of our collaborated research groups, your institution will be informed for this and strict legal actions will be taken immediately.)
- To guard yourself and others from possible illegal use please do not permit anyone right to use to your paper and files.

Criterion for Grading a Research Paper *(Compilation)* by Global Journals

Please note that following table is only a Grading of "Paper Compilation" and not on "Performed/Stated Research" whose grading solely depends on Individual Assigned Peer Reviewer and Editorial Board Member. These can be available only on request and after decision of Paper. This report will be the property of Global Journals.

Topics	Grades		
	A-B	**C-D**	**E-F**
Abstract	Clear and concise with appropriate content, Correct format. 200 words or below	Unclear summary and no specific data, Incorrect form Above 200 words	No specific data with ambiguous information Above 250 words
Introduction	Containing all background details with clear goal and appropriate details, flow specification, no grammar and spelling mistake, well organized sentence and paragraph, reference cited	Unclear and confusing data, appropriate format, grammar and spelling errors with unorganized matter	Out of place depth and content, hazy format
Methods and Procedures	Clear and to the point with well arranged paragraph, precision and accuracy of facts and figures, well organized subheads	Difficult to comprehend with embarrassed text, too much explanation but completed	Incorrect and unorganized structure with hazy meaning
Result	Well organized, Clear and specific, Correct units with precision, correct data, well structuring of paragraph, no grammar and spelling mistake	Complete and embarrassed text, difficult to comprehend	Irregular format with wrong facts and figures
Discussion	Well organized, meaningful specification, sound conclusion, logical and concise explanation, highly structured paragraph reference cited	Wordy, unclear conclusion, spurious	Conclusion is not cited, unorganized, difficult to comprehend
References	Complete and correct format, well organized	Beside the point, Incomplete	Wrong format and structuring

Index

P

R

S

T

U