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LETTER TO THE EDITOR

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Online version at http://www.minervamedica.it
Panminerva Medica 2020 mese;62(0):000–000
DOI: 10.23736/S0031-0808.19.03588-2

Can paranasal sinus computed tomography screen for cystic fibrosis heterozygotes?

We would like to introduce a method that could simplify the identification of heterozygotes carriers of cystic fibrosis transmembrane conductance regulator (CFTR) mutations, then improving the sensitivity of the genetic evaluation, and defining the etiology of chronic sinusitis in selected cases.

Since the identification of the CFTR on chromosome 7, back in 1989, more than 1893 different mutations have been identified and grouped to six "classes" according to the effect induced on the CFTR protein.[1] Considering the costs of genetic analysis, a general, systematic, screening for CFTR mutations of the general population is still untenable in order to identify CFTR mutations carriers. While cystic fibrosis (CF) patients can be identified by CF symptoms (which can be severe or mild, depending on the combinations of different mutations on two alleles),[2] CF heterozygotes carriers do not show specific clinical patterns, apart a higher prevalence of chronic rhinosinusitis, as previously reported.[3] Hence, considering the radiological features described in few CF heterozygotes carriers (thin, diffused thickening of nasosinusal mucosa without inflammatory polyps, at the sinonasal CT scan), we performed a study on a large number of sinus-nasal CT scans, aiming to identify patients with similar CT findings. Patients exclusively selected on these radiological criteria, underwent genetic testing for mutations in the CFTR gene.

A total of 5292 consecutive sinonasal CT scans performed between January 1st, 2010 and December 31st, 2015 at our University setting were retrieved and studied retrospectively.

Inclusion criteria were: 1) adult patients, aged between 18 and 60 years; 2) presence of a symmetric and bilateral chronical sinusitis, affecting at least two or more sinus districts among maxillary, ethmoidal, sphenoid and frontal sinuses; 3) presence of a mucosal homogenous thickening of at least 4 mm (as per the sinonasal CT scan findings of CF heterozygotes carriers previously identified) (Figure 1).

Exclusion criteria were: 1) grade 3 and 4 of sinus polyps according to Lund-Kennedy; 2) recent trauma and/or pseudo-sinusopathy derived from trauma (such as hemo-sinuses); 3) radiological evidence of anatomic anomalies which could induce sinusitis (like concha bullosa, septal deflection).

Only 101 patients fully satisfied the above-mentioned criteria, and then were invited, by letter, to participate to an interview. At this stage, after history taking, we further excluded subjects with a history of drug use/abuse and history of smoking: finally, a total of 62 subjects were selected for genetic testing, and agreed to participate to the study.

All 62 subjects underwent a complete ENT evaluation (resulting normal in all cases) and therefore a venous blood sample was taken for genetic analysis of CFTR gene.

Genomic DNA was extracted by leukocytes present in peripheral blood sample, using an automatic procedure with a BioRobot Universal System© provided by QIAGEN. This instrument uses a DNA amplification through a polymerase chain reaction (PCR) technique. The process normally uses fragments of 15-25 nucleotides (called primer) complementary to the sequence to be amplified. (coding helix and anti-coding helix respectively). The first level of research is the reverse dot blot. This technique provides a multiplex PCR, which is able to amplify different gene regions. The analysis is fast and reliable and is performed by an instrumental kit (INNOLIPA) which is able to locate the most frequent mutations of CFTR gene. For a more detailed study, our samples were also examined for specific genetic alterations (second level analysis) through a chromatography test (DHPLC). This methodology allows to identify any mismatches between nucleotides, caused by replacements, little insertions or deletions, regardless of their own characteristics and fragment location. PCR extracts underwent chromatography using a specific technique device called WAVE 4500 Genetics Analysis System. The results are analyzed by means of a specific software; all samples showing

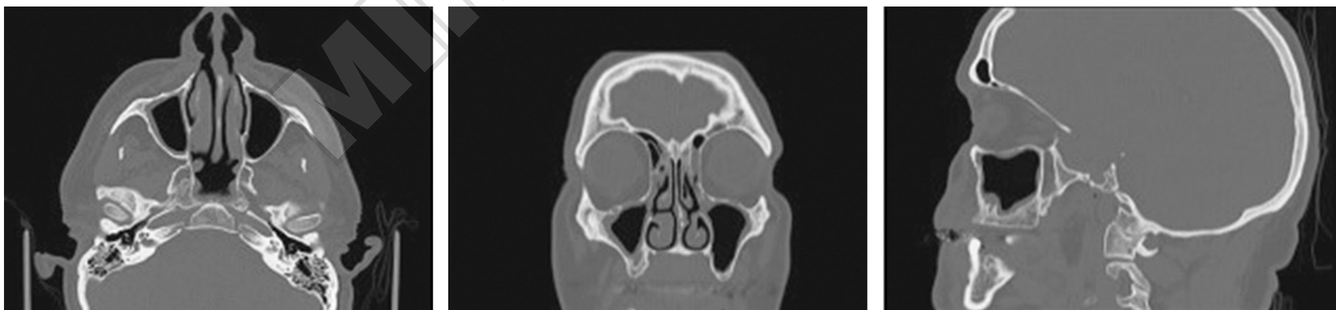

Figure 1.—Mucosal homogenous thickening of at least 4mm in both maxillary sinuses, in a CF heterozygotes carrier (axial, coronal and sagittal scans).

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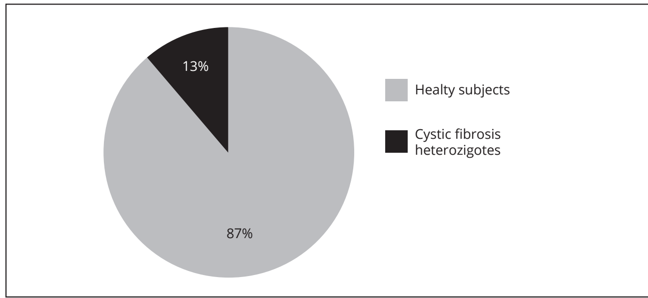

Figure 2.—Prevalence of cystic fibrotic heterozygotes among the 62 subjects analyzed.

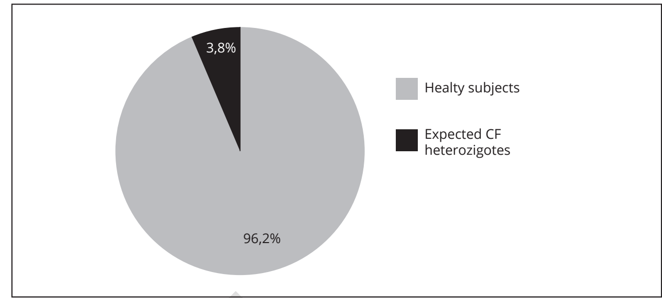

Figure 3.—Expected prevalence of cystic fibrotic heterozygotes among the 62 subjects analyzed.

TABLE I.—*Pathological mutations of cystic fibrosis.*[5]

Patient #	Mutation			Position	Consequences	Clinical meaning
	cDNA name (international nomenclature)	Protein name	Legacy name			
23	c.2991G>C	p.Leu997Phe	L997F	Exon 19	Leucine or phenylalanine in 997 position	Atypical cystic fibrosis
34	c.509G>A	p.Arg170His	R170H	Exon 5	Guanine instead of adenosine in 641 position	Mutation found in a 48-year-old man investigated for infertility, in association to F508 mutation; it was also found in a 12-year-old boy, subject of a genetic consultation. Both had a F508 deletion
36	c.3472C>T	p.Arg1158X	R1158X	Exon 22		Pathological mutation
51	c.91C>T	p.Arg31Cys	R31C	Exon 2	Change in nucleotide from cytosine to thymine in 223 position on exon 2, this produces a cysteine instead of arginine, on codon 31 (R31C)	Missense mutation on CFTR gene; was found for the first time in a 35-year-old patient affected by atypical form of CF. Dubious functional meaning
57	c.3469-65C>A	-	3601-65C/A	Intron 21	Cysteine or adenine in Al 3601-	Found on patients affected by disseminated bronchiectasis. Dubious functional meaning
62	c.91C>T	p.Arg31Cys	R31C	Exon 2	Change in nucleotide from cytosine to thymine in 223 position on exon 2, this produces a cysteine instead of arginine, on codon 31 (R31C)	Missense mutation on CFTR gene; was found for the first time in a 35-year-old patient affected by atypical form of CF. Dubious functional meaning

TABLE II.—*Mutations that are not causative of cystic fibrosis.*[5]

Patient #	Mutation			Position	Consequences	Clinical meaning
	cDNA name (international nomenclature)	Protein name	Legacy name			
21	c.224G>A	p.Arg75Gln	R75Q	Exon 3	Substitution of arginine (polar amino acid) with glutamine (neuter amino acid) on exon 3, which codify for the transmembrane region of CFTR channel called MSD1	Mutation on amino acid n. 75, does not cause cystic fibrosis even if present in concomitance with another pathogenic mutation of CFTR gene
44	c.224G>A	p.Arg75Gln	R75Q	Exon 3	Substitution of arginine (polar amino acid) with glutamine (neuter amino acid) on exon 3, which codified for the transmembrane region of CFTR channel called MSD1	Mutation on amino acid n. 75, does not cause cystic fibrosis even if present in concomitance with another pathogenic mutation of CFTR gene

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an anomalous profile compared to the “wild type” DNA were considered positive.[4]

Informed written consent was obtained from all participants. The study was approved by the Ethical Committee (April 16th, 2015 – No. 150295).

The Pearson χ^2 test was used to evaluate the clinical data. The level of significance was set at $P<0.05$. The data were analyzed using SPSS v. 17 (IBM Corp., Chicago, IL, USA).

Among the 62 patients analyzed, 24 were males and 38 females (38.71% and 61.29%, respectively), average age of 41.59 years (range 18-60 years); eight were identified to be carriers of CFTR gene pathological mutations. Eight subjects of 62 correspond to a prevalence of 12.9% of cystic fibrosis heterozygotes in the selected cohort, instead of the 3% expected in the Italian general population (Figure 2, 3).

As the estimated prevalence of CF heterozygotes in the Italian population, according to Italian Society of Cystic Fibrosis, is one in 26 (q=0.038), we expected to find 2.35 CF heterozygotes among the 62 subjects. Nonetheless, comparing the expected value (2.35/62 CF heterozygotes) and the real value found (eight of 62 CF heterozygotes), the index value obtained in the studied population is 3.77 ($P<0.001$).

Among the specific genetic mutations found, 6 can be considered as pathological causes of CF (Table I). The other two mutations found are reported not to be causative of CF (Table II).

Our results show that selecting patients on typical sinonasal CT scans patterns may help in identify CF heterozygotes carriers. In particular, this methodology could: 1) save resources, defining a cohort of subjects for genetic testing; 2) increase the total number of subjects identified as CF heterozygotes affected by chronic sinusitis.

However, the present study cannot be considered a new screening method for a universal identification of CF heterozygotes. This method can help, in the daily practice, physicians and Otorhinolaryngogists to recognize heterozygosis CF carriers, particularly distinguishing them from the general population, affected by chronic sinusitis. In the future, it will be interesting to perform greater studies on larger cohorts of heterozygotes; it is likely that, if costs of genetic testing would lower, it could be possible to access these exams more easily.

Nicola MALAGUTTI [1], Cristina COGLIANDOLO [1], Doriana FRANCIOSI [1], Alessandra FERLINI [2], Paola RIMESSI [2], Anna RAVANI [2], Chiara BIANCHINI [1], Andrea CIORBA [1] *, Francesco STOMEO [1], Stefano PELUCCHI [1]

[1]Department of Ear Nose and Throat, University Hospital of Ferrara, Ferrara, Italy; [2]Unit of Medical Genetics, Department of Medical Sciences, University Hospital of Ferrara, Ferrara, Italy

*Corresponding author: Andrea Ciorba, Department of Ear Nose and Throat, University Hospital of Ferrara, Ferrara, Italy. E-mail: andrea.ciorba@unife.it

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Conflicts of interest.—The authors certify that there is no conflict of interest with any financial organization regarding the material discussed in the manuscript.

History.—Article first published online: February 11, 2019. - Manuscript accepted: February 4, 2019. - Manuscript revised: January 25, 2019. - Manuscript received: December 4, 2018.

(*Cite this article as:* Malagutti N, Cogliandolo C, Franciosi D, Ferlini A, Rimessi P, Ravani A, *et al*. Can paranasal sinus computed tomography screen for cystic fibrosis heterozygotes? Panminerva Med 2020;62:000–000. DOI: 10.23736/S0031-0808.19.03588-2)

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