MEDICINA y SEGURIDAD del trabajo

Revisiones

Genetic polymorphism linked to the probability of developing occupational asthma in workers exposed to isocyanates

Polimorfismo genético relacionado con la probabilidad de desarrollar asma ocupacional en trabajadores expuestos a isocianatos

Gaetano Pepe Betancourt [1,4], Tonny Hibsen Da Silva Sánchez [2,5], Ivanio Godoy Carus [3,6]

1. *Hospital San Pedro. Logroño. España.*
2. *Complejo Universitario Hospitalario de Vigo. Galicia. España.*
3. *Hospital Severo Ochoa. Madrid. España.*
4. *Unidad Docente de Medicina del Trabajo de La Rioja. España.*
5. *Unidad Docente de Medicina del Trabajo de Galicia. España.*
6. *Unidad Docente de Medicina del Trabajo de la Comunidad de Madrid. España.*

Recibido: 23-01-14
Aceptado: 19-02-14

Correspondencia
Gaetano Pepe Betancourt
Servicio de Prevención de Riesgos Laborales
Hospital San Pedro
C/ Piqueras 98
CP 26007 Logroño (La Rioja) España
Tfno: 941298390
E-mail: gpbetancour@riojasalud.es

Este trabajo se ha desarrollado dentro del Programa Científico de la Escuela Nacional de Medicina del Trabajo del Instituto de Salud Carlos III en Convenio con la Unidad Docente de Medicina del Trabajo de La Rioja, Unidad Docente de Medicina del Trabajo de Galicia y la Unidad Docente de Medicina del Trabajo de la Comunidad de Madrid.

Abstract

Introduction: Technological development has led to the use of chemicals potentially damaging to workers' health. In particular, the use of isocyanates has resulted in a greater morbidity caused by respiratory pathology, especially asthma. Considering that not all the exposed workers develop the disease, a gene-environment interaction model has been proposed, which tries to explain the genetic predisposition that some individuals have and others have not to develop occupational asthma.

Objective: To find out about the scientific evidence related to the genetic polymorphism and workers exposed to isocyanates' susceptibility to develop occupational asthma.

Methods: We conducted a systematic review. Bibliographic electronic searches were conducted in PubMedline databases, as well as in Dialnet and ELSEVIER repositories. Papers related to this review's objective were retrieved, without applying temporary filters, using the following descriptors: MeSH Major Topic, MeSH Terms. Search period started on November 20th 2013 and ended on December 15th 2013. We established the level of evidence according to GRADE criteria.

Results: Forty-two full-text papers were analyzed, scientific evidence being supported by eleven case-control studies. Given the complexity of the genetic polymorphism associated with disease phenotype, as a

limitation from the studies, authors agree that the sample size is not large enough, yet the papers found obtained a GRADE B level of evidence after adjustment for confounding factors.

Conclusion: Genetics has a significant influence on isocyanate-induced occupational asthma. The weight of genetic susceptibility and gene-environment interaction remains unclear. Understanding these relationships has implications for workers' health, given the influence that some workplace factors have on genetic risk, which can and must be modified.

Med Segur Trab (Internet) 2014; 60 (234) 260-280

Key words: *occupational asthma, isocyanates, susceptibility, genetic polymorphism.*

Resumen

Introducción: El desarrollo tecnológico ha traído como consecuencia el uso de sustancias químicas potencialmente perjudiciales para la salud de los trabajadores. Particularmente el uso de isocianatos ha resultado en una mayor morbilidad de patología respiratoria, especialmente el asma. Considerando que no todos los trabajadores expuestos desarrollan la enfermedad se ha propuesto un modelo de interacción gen-medioambiental, el cual trata de explicar la predisposición genética que tienen algunos individuos a desarrollar asma ocupacional y otros no.

Objetivo: Conocer la evidencia científica relacionada con el polimorfismo genético y la susceptibilidad que tienen los trabajadores expuestos a isocianatos a desarrollar asma ocupacional.

Metodología: Se realizó una revisión sistemática mediante una búsqueda bibliográfica utilizando las bases de datos PubMedline, así como en los repositorios Dialnet y ELSEVIER. Se extrajeron los artículos relacionados al objetivo de esta revisión, no se aplicaron filtros de temporalidad, utilizándose los siguientes descriptores: MeSH Major Topic, MeSH Terms. El periodo de búsqueda fue desde el 20 de noviembre 2013 y finalizo el 15 de diciembre del 2013. El nivel de evidencia se estableció de acuerdo a los criterios GRADE.

Resultados: Se analizaron a texto completo 42 artículos, la evidencia científica se sustentó en 11 estudios de casos-controles. Dada la complejidad del polimorfismo genético asociado con la expresión fenotípica de la enfermedad, como limitación de los estudios, los autores coinciden que el tamaño muestral no es suficientemente grande, sin embargo después de ajustar los factores de confusión los artículos encontrados tuvieron un nivel de evidencia B de GRADE.

Conclusión: La genética tiene una influencia significativa en el asma ocupacional inducida por isocianatos. El peso de la susceptibilidad genética y de la interacción gen-medioambiente aún no se han esclarecido del todo. Comprender estas relaciones tiene implicaciones para la salud de los trabajadores, dado que algunos factores del lugar de trabajo tienen influencia en el riesgo genético los cuales pueden y deben modificarse.

Med Segur Trab (Internet) 2014; 60 (234) 260-280

Palabras claves: *Asma ocupacional, isocianatos, susceptibilidad y polimorfismo genético.*

INTRODUCTION

Technological and industrial development has led to the use of various chemicals. More than 250 organic and inorganic substances have been identified, smoke, dust, vapors, etc., capable of causing occupational respiratory pathologies, being occupational asthma (OA) the most common pathology [1,2]. According to the WHO, 217 million occupational disease cases occur each year, 50 million corresponding to respiratory pathology and 20-40% of them correspond to OA. It is important to note that in industrialized countries 9-15% of physician-diagnosed asthma cases in adults are related to work environment and that isocyanates (NCO) are responsible for 5-10% of this disease [1,14]. According to CEPROSS' statistical data [3] 24 cases of occupational disease caused by isocyanates have been reported from January to November 2013. It's been reported that painters from automotive industry who use isocyanate-based paints are 80 times more likely to suffer OA than other workers (4) and attributable risk ranges from 3% to 7%.

Occupational asthma is characterized by variable airflow obstruction and/or by airway hyperreactivity due to exposure in the workplace and can account for 10%-25% of adult asthma cases. Epidemiologically more than 90% of occupational asthma cases are induced by bronchial hyperreactivity phenomena [5]. It has been reported that some of the individuals who develop occupational asthma by NCO remain with asthma symptoms ever after cessation of exposure. Paggiaro et al. observed asthma remission only in 50% of subjects after quitting their jobs. The unfavorable development in half of the patients is mainly attributed to long duration of symptoms before diagnosis, as well as greater severity of it [2]. Furthermore, it is a preventable disease, so protection measures must be implemented in those sectors where these substances are manipulated, protecting and promoting worker health.

Isocyanates are highly reactive, low molecular weight aliphatic and aromatic compounds, being the airways the main routes of exposure, mainly vapor and aerosol inhalation, although exposure may also occur through skin contact during handling of liquid or airborne isocyanates. Regarding exposure levels, permissible environmental acute exposure limit value (VLA-ED) for phenylisocyanate (MDI) (0.01 ppm), 3-Isocyanatomethyl-3,5,5-trimethylcyclohexyl isocyanate (TDI) (0.05 ppm) and for methyl isocyanate (HDI) permissible environmental chronic exposure limit value VLA-EC (0.02), VLA-ED not established for the latter. These compounds are increasingly being used and nowadays a lot of workers are exposed to complex mixtures of isocyanate oligomers [6].

Physiopathological mechanisms causing asthma induced by NCO have multiple etiologies. Diagnosis, evolution and prognostic depend on time, exposure concentration and seniority in the workplace, as well as on predisposing factors such as airways hyperreactivity, genetic predisposition and smoking. With regard to the latter factor, risk for occupational asthma after exposure to occupational allergens is higher among smokers and it seems that tobacco promotes sensitization. In addition, it is pointed out that methyl isocyanate (MDI) is a substance found among tobacco components so that workers are more exposed to it.

With respect to immunological mechanism, HLA class II molecule loci are found in the short arm of chromosome 6 (6p21.3) and they play a crucial role in immune response, as they bind antigen derived peptides and present them to T cells through T cell receptor, the IgE mediated mechanism is the most involved mechanism and so far the most studied [7,8,9,10]. Moreover, genetic predisposition is important, since probably sensitization against allergens present in workplace is conditioned by specific genetic factors and restricted by HLA antigens, which may explain at least in part why certain individuals develop OA and others not. On the other hand, not always the employee withdrawal from workplace was followed by a complete remission of symptoms, in fact this has occurred in only a small percent of the cases (24.3%) [11]. Recently, associations between specific HLA phenotypes and sensitization against certain occupational allergens have been described, as well as protective factors and the influence of modifier genetic factors

of OA induced by NCO susceptibility [12]. NCO metabolism is not yet understood, but there is evidence that multiple metabolic pathways are involved in the biotransformation [13].

Biological monitoring is increasingly used to evaluate exposure and control measures efficiency [4-14]. Biological detection methods are already available for most common NCO, based on analysis of hexamethylene diamine, toluene diamine, isophorone diamine, and methylenediamine released after isocyanate-protein adducts' hydrolysis in urine or blood and whose metabolite in urine in hippuric acid [15]. Occupational studies show a good correlation between exposure through inhalation to isocyanate monomers and isocyanate-derived diamines in urine or blood. However, occupational exposure to NCO is often a mixture of monomers and oligomers so there is some uncertainty in the comparison of the results of the biological control of exposure [4-14]. Biological markers have relatively short half-lives in urine, 2-5 hours, which means that urine samples must be collected at the end of exposure and results mainly reflect exposure on the day of collection [14], half-lives in plasma are usually longer (20-25 days), Sabbioni et al. have shown that methylenediphenyl diisocyanate (MDI) forms adducts with lysine in plasma albumin and may offer a potential for assessing risk [16-17]. All the volunteer studies and occupational studies with both air and biological monitoring show good correlations between exposure, mostly inhalation, and levels of biomarkers in urine or blood and advocate the usefulness of biological monitoring. Nevertheless, the convenience and non-invasive nature of urine sampling make it the most widely used approach for assessing exposure [16]. There is a clear evidence of dermal exposure and absorption [18,19], but dermal exposure is variable and depends largely on the exposure process, NCO volatility and on the use of appropriate personal protective measures (PPE).

OBJECTIVE

This review aims to asses the scientific evidence that relates genetic polymorphism and workers exposed to isocyanates susceptibility of developing occupational asthma.

SPECIFIC OBJECTIVES:

1. To acknowledge the relationship between genetic polymorphism and possible physiopathological mechanisms of asthma induced by isocyanates.
2. To describe the presence of a specific gene and/or his allele, as well as its modification with the clinical manifestations of asthma induced by isocyanates.
3. To identify the presence of protective genes or inducers of susceptibility of developing asthma by isocyanates.

METHODS

We conducted a paper search in bibliographic databases on the Internet, PubMed/MEDLINE, ELSEVIER, SciELO, Google Scholar. To conduct the search free terms and descriptors were used DeCS, MeSH and MeSH Major Topic. Addressing the issue of workers with asthma related to exposure to isocyanates and the association between the disease and genetic polymorphism. Search period started on November 20th 2013 and ended on December 15th 2013. Search formulas are shown in Table 1.

Table 1. Search strategy

Database	Search terms	Search strategy
MEDLINE-Pubmed	Isocyanates, metabolism, urinary excretion, adverse effects, asthma and genetic polymorphism.	("isocyanates/metabolism"[MeSH Terms]) AND "isocyanates/urine", ("isocyanates/metabolism"[MeSH Terms]) AND "isocyanates/adverse effects"[MeSH Terms] Filters: published in the last 10 years; Humans, (("Isocyanates"[Majr]) AND Asthma[MeSH Terms]) AND "genetic variation"[MeSH Terms] (("isocyanates"[MeSH Major Topic]) AND "asthma"[MeSH Terms]) AND "polymorphism, genetic"[MeSH Terms].
Dialnet	Isocyanates, metabolism, urinary excretion, adverse effects, asthma and genetic polymorphism.	Isocyanates, metabolism AND, urine excretion AND, sampling AND asthma AND genetic polymorphism.
ELSEVIER	Isocyanates, metabolism, urinary excretion, adverse effects, occupational asthma and genetic polymorphism.	Isocyanates, metabolism AND, urine excretion AND, sampling AND asthma AND genetic polymorphism.
Academic Google	Isocyanates, metabolism, asthma and genetic polymorphism.	asthma AND humans AND exposition AND Isocyanates AND bronchial hyperreactivity AND metabolism AND genetic polymorphism AND adverses effects filetype:pdf.

Once obtained the bibliographic collection, relevance assessment was carried out by comparing the suitability of the papers for the study from the review of titles and abstracts.

Subsequently, the following inclusion and exclusion criteria were applied to selected papers.

Table 2. Inclusion and exclusion criteria

Inclusion criteria	Exclusion criteria
Observational cohort and case control studies.	Non-occupational asthma.
Context: asthma as an occupational disease and the association with exposure to isocyanates, polymorphism and genetic susceptibility.	Toxicokinetics.
National and international studies.	Clinical cases, concerning one case.
English, Spanish, Italian or Portuguese language.	—

1. In case of finding a paper repeated in several publications, the one included was that one published in the journal with the highest impact factor and/or the most recently published.
2. Books, protocols, reviews, communications, technical notes.

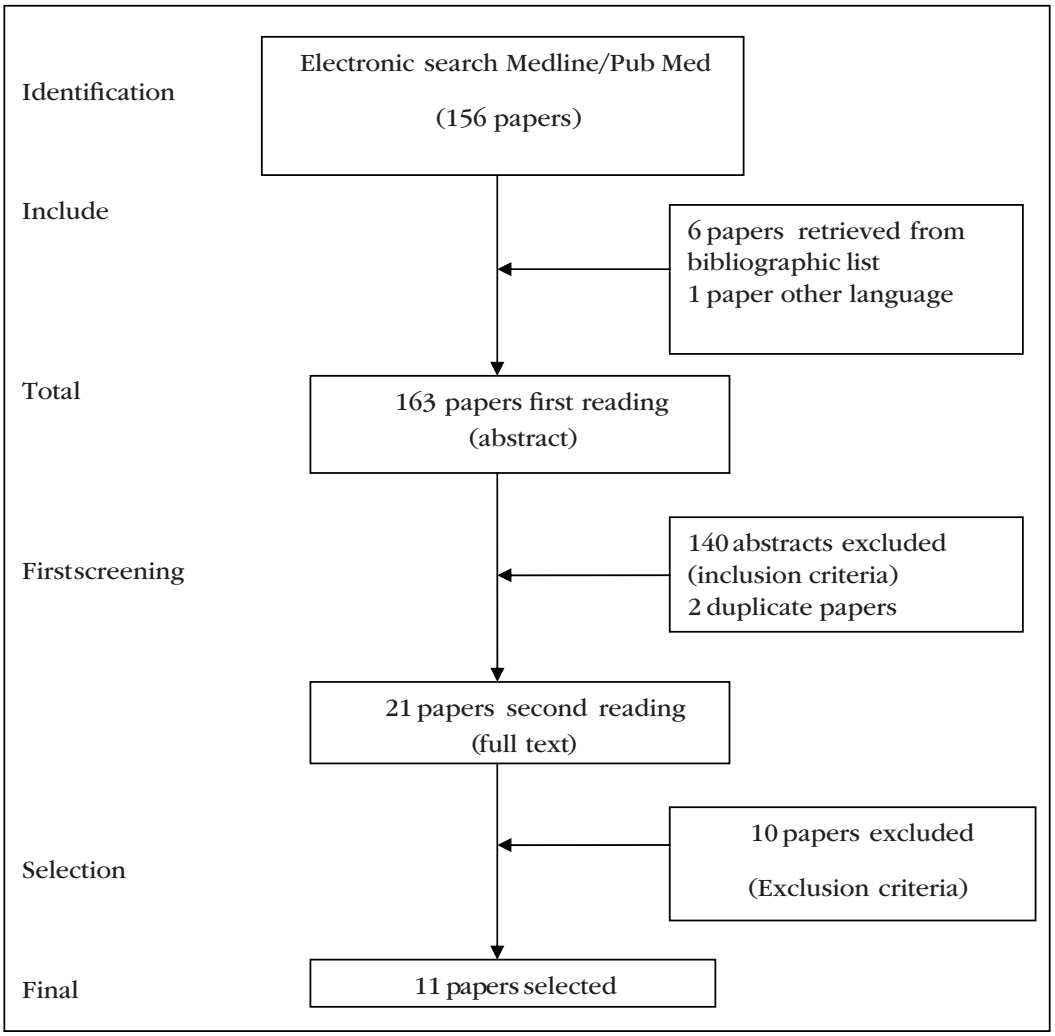
Paper identification was concluded with the search of full text bibliographical material from the preselected works, after an initial screening. For the evidence synthesis, in every paper we analyzed: study type, sample size and type, confounding factors, bias control measures, dependent variables and measurement techniques, independent

variables and assessment techniques, results and results measure and conclusions. Any discrepancies were solved by consensus among the team components.

RESULTS

Based on the search strategy, a total of 156 articles were obtained, after eliminating duplicate papers and the items from the reference list included, we proceeded to the analysis of relevance and application of the inclusion and exclusion criteria, finally obtaining 11 items that form the basis of this study (Figure 1).

Figure 1. The flowchart illustrates the procedure followed and the results obtained in paper selection, once applied the inclusion and exclusion criteria.



All the selected papers corresponded with observational studies, case-control longitudinal studies and longitudinal ambidirectional cohort studies, with bias control through logistic regression analysis, the largest of them with a sample size of 410 subjects. Level of evidence: B GRADE.

Even without applying temporary filters to paper search, we observed that the largest number of papers were published between 2004 and 2013 (54.5%), the first one published by A. Balboni in 1996.

We can find the most prolific scientific production in Europe with a total amount of 7 papers (63,63%), followed by Canada and The United States, with 3 items (27.27%) being collaborative studies among which Spain is found.

90.9% of the studies (10 papers), were conducted with workers suffering asthma confirmed through methacholine test and FEV1. Only one of the studies was made based on symptoms (eye irritation, runny nose, sneezing, dry or productive cough), the exposure to isocyanates in monomeric and oligomeric its various forms was always the standard, as well as asymptomatic asthmatic control cases exposed, non exposed and non asthmatic controls.

Main features and results from reviewed articles are shown in Table 2, which summarizes the main peculiarities of each one in terms of author, year and country of publication, title of the study, type of design, population and sample.

Table 3. Features from reviewed articles

Author	Year	Paper	Type of study	Population	Sample	Bias control
A. Balboni	Italy 1996	Association between toluene diisocyanate-induced asthma and DQB1 markers: a possible role for aspartic acid at position 57.	Cases and controls	Caucasian North of Italy	N=168	Yes
C. E. Mapp	Italy 2000	Association between HLA genes and susceptibility to toluene diisocyanate-induced asthma.	Cohort prospective ambidirectional	Caucasian North of Italy	N=195	Yes
PaÈ ivi Piirilaç	Finland 2001	Glutathione S-transferase genotypes and allergic responses to diisocyanate exposure.	Cases and controls	Workers exposed to HDI, MDI, TDI	N=182	Yes
Cristina E	Italy 2002	Glutathione S-transferase *GSTP1* is a susceptibility gene for occupational asthma induced by isocyanates.	Cases and controls	Workers exposed to TDI	N=131	Yes
Harriet Wikman	Finland France 2002	N-Acetyltransferase genotypes as modifiers of diisocyanate exposure-associated asthma risk.	Cases and controls	Workers exposed to HDI, MDI, TDI	N= 182	Yes
B. Begh	Italy 2004	Lack of association of HLA class I genes and TNF a-308 polymorphism in toluene diisocyanate-induced asthma.	Cases and controls	Workers exposed to TDI	N=192	Yes
David I. Bernstein	Canada USA Spain 2006	Diisocyanate asthma and gene-environment interactions with *IL4RA*, *CD-14*, and *IL-13* genes.	Cases and controls	Workers exposed to isocyanates	N=137	Yes
Karin Broberg	Sweden 2008	Influence of genetic factors on toluene diisocyanate-related symptoms: evidence from a cross-sectional study.	Cases and controls	Workers exposed to TDI	N=246	Yes
Jeong-Hee Choi	Korea 2009	The HLA DRB1 * 1501-DQB1 * 0602-DPB1 * 0501 Haplotype Is a Risk Factor for Toluene Diisocyanate-Induced Occupational Asthma.	Cases and controls	Workers exposed to TDI	N=258	Yes
Berran Yucesoy	Canada France 2012	Genetic Variants in Antioxidant Genes Are Associated With Diisocyanate-Induced Asthma.	Cases and controls	Workers exposed to HDI, MDI, TDI	N=353	Yes
David I. Bernstein	Canada USA Spain 2012	(CTNNA3 (α-Catenin) Gene Variants Are Associated With Diisocyanate Asthma: A Replication Study in a Caucasian Worker Population.	Cases and controls	Workers exposed to HDI, MDI, TDI	N= 410	Yes

High complexity of HLA class II genotype make the studies to be very complicated and sometimes with contradictory results, however the role of this polymorphism in asthma pathogenesis is known, A. Balboni et al.[20] confirm that gene DQB1 *0503 is associated to susceptibility in so far as the DQB1 * 0501 is associated to protection, but both genes are associated to susceptibility and the protective nature is provided only through the substitution by Aspartic amino acid in position 57 in * 0503 and Valine in * 0501, condition most frequently observed in homozygous, suggesting a direct role for this residue in the etiology of asthma induced by toluene. Other HLA class II genes were studied by C. E. Mapp et al.[8] This study shows a positive association of DQA1 * 0104 and DQB1 * 0503 with the disease and a negative association with DQA1 * 0101 y DQB1 * 0501, which suggest that DQA1 and DQB1 are markers involved in susceptibility and protection of asthma induced by TDI (Table 4).

The predominant form of occupational exposure to diisocyanates is through inhalation pathway, however dermal exposure is still important; Thomasen JM et al.[18], observed that dermal exposure is linked to the individual skin penetration rate of isocyanate. On the other hand, Arrandale V et al.[19], observed a statistically significant association between dermal absorption, athopy and respiratory symptoms. Reactive isocyanate compounds form covalent conjugates mediated by N-acetyltransferase system (NAT) because genotypes for slow acetylators and their combination (NAT2/NAT1 slow acetylator and null GSTM1, represented a greater risk of suffering asthma induced by isocyanates, specially for toluene, fact observed by Harriet W. et al.[13] Furthermore the formation of leukotrienes has been reported as potential mediators of histamine response, also responsible for the pathogenesis of bronchial asthma, the formation of these metabolites depends largely on N-acetylation by GST, playing a significant role in modulation of individual susceptibility to exposure associated to diisocyanates and occupational asthma risk (Table 5).

Up to date, common allelic variants have been found in four genes from Glutathione S-transferase (GST), when the genetic polymorphism was studied, the most marked effect was seen with the combination of genotypes GSTM1 null and GSM3 AA with lack of IgE specific antibodies and late change in the provocation tests, in Päivi Piirilä's study (21) no statistically significant association was observed between the genotypes GSTP1 and the risk of asthma induced by diisocyanates, so they suggest that the polymorphism for GST plays an important role modulating the response against exposure to diisocyanates. Other authors as Cristina E. (22) observed statistically significant association only for the gene GSTP1 * Val105, which confers protection against asthma induced by TDI and airway hyperreactivity. This view is supported by the finding that the protective effect increase in proportion to the duration of exposure to TDI (Table 6).

Table 4. Association of HLA class II polymorphism, associated to the risk of OA induced by isocyanates

Author	Paper	Type of study	Population and sample	Level of evidence GRADE	Genetic polymorphism studied	Measures of association	Results, discussion and conclusions
A. Balboni. Italy 1996	Association between toluene diisocyanate-induced asthma and DQB1 markers: a possible role for aspartic acid at position 57.	Cases and controls	Caucasian North of Italy **N=168**	B	HLA II BQB1 0503 Asp/Asp57 0501 Val57	 p=0.0447 RR: 5,58 pc=0.038 RR: 0,04	The evaluation of HLA class II genes in cases of asthma induced by TDI showed a positive association with HLA-DQB1 * 0503 (susceptibility) and a negative association (protection) with HLADQB1 * 0501 alleles, which differed on residue 57 for only one aminoacid, namely aspartic acid on DQB1 * 0503 and DQB1 * valine in 0501.
C. E. MAPP Italy 2000	Association between HLA genes and susceptibility to toluene diisocyanate-induced asthma.	Cohort prospective ambidirectional 10 years	Caucasian North of Italy **N=195**	B	HLA II DQA1 0104 DQB1 0503 Aspartic57* DQA1 0101 Valine57* DQB1 0501	 p=0.008 p=0,027 p=NS p=NS	This study shows a positive association of DQA1 * 0104 and DQB1 * 0503 with the disease and a negative association with DQA1 * 0101 and DQB1 * 0501, which suggest that markers DQA1 and DQB1 are involved in susceptibility and protection for asthma induced by TDI. Most of the asthmatic subjects were homozygous for the presence of a residue of aspartic acid on position 57, which suggest a direct role for this residue in the etiology of this type of occupational asthma. It was also noted that subjects could develop asthma induced by isocyanates from 1-2 years of exposure.

Table 5. Genetic polymorphism of N-acetyl transferase (NAT) associated to and TDI combination of isocyanates

Author	Paper	Type of study	Population and sample	Levelof evidence GRADE	Genetic polymorphism studied	Measures of association	Results, discussion and conclusions
Harriet Wikman Finland France 2012	N-Acetyltransferase genotypes as modifiers of diisocyanate exposure-associated asthma risk.	Cases and controls	Workers exposed to HDI, MDI, TDI **N= 182**	B	NAT1		The genotypes for slow acetylators, posed a greater risk of suffering asthma induced by isocyanates, especially for toluene, with lower risk when workers are exposed to polymeric forms of isocyanates TDI, MDI, HDI. The NAT pathway might also affect the formation of immunological haptens from isocyanates. Hapten formation would be more favored in slow acetylators than in rapid acetylators.
					GSM1 null	OR 4.53 (1.76-11.6)	
					RAPID ACETYLATORS		
					Alleles 10/11		
					SLOW ACETYLATORS		
					Alleles 3/4/14[a]/15		
					TDI	OR 7.7 (1.18-51.6)	
					TDI/MDI/HDI	OR 2.54 (1.32-4.91)	
					NAT2		
					GSM1 null	OR 3.12 (1.11-8.78)	
					COMBINACION		
					NAT1/NAT2 SLOW AC.	OR 4.20 (1.51- 11.6)	
					GSM1 null/NAT1 MDI	OR: 7.58 (1.42-40.3)	
					GSM1 null/NAT1 TODO	OR: 4.53 (1.76-11.6)	
					GSM1 null /NAT2 MDI	OR:7.34 (1.33-40.4)	
					GSM1 null/NAT2 TODO	OR:3.12 (1.11-8.78)	

Table 6. Association of GST genotypes and combination of genotypes GSTM1 null and GSTM3 AA

Author	Paper	Type of study	Population and sample	Level of evidence GRADE	Genetic polymorphism studied	Measures of association	Results, discussion and conclusions
Päivi L Piirilä Finland 2001	Glutathione S-transferase genotypes and allergic responses to diisocyanate exposure.	Cases and controls	Workers exposed to HDI, MDI, TDI **N=182**	B	Genes of Glutathione S-transferase GSTM1 Null GSTM3 AA COMBINATION GSTM1-GSTM3	 OR 1.89 (1.01±3.52) OR 3.75 (1.26-11,2) OR 11.0 (2.19-55.3)	Association of the combination GSTM1-GSTM3, observing lower levels of specific IgE-diisocyanate, but greater clinical symptomatology, which might suggest that diisocyanates can react with Glutathione S-transferase to form conjugates, which are excreted as such or forming haptens which decrease when the amount of conjugates of GST-diisocyanate is reduced due to deterioration of the activity of the enzyme GST, translating into modulating activity to the formation of specific IgE.
Cristina E Italy 2002	Glutathione S-transferase *GSTP1* is a susceptibility gene for occupational asthma induced by isocyanates.	Cases and controls	Workers exposed to TDI **N=131**	B	Genes of Glutathione S-transferase Homozygous Valine *GSTP1* *Ala114/Ala114* AHR negative AHR Positive Asthma negative Asthma positive	 P =.033 P= 0,2 OR 0.23 (0.05-1.13)	These data suggest that homozygosity for GSTP1 * Val105 allele confers protection against asthma induced by TDI and airway hyperreactivity. This view is supported by the finding that the protective effect increases in proportion to the duration of exposure to TDI.

Table 7. Genetic variants of the GST, manganese superoxide dismutase (SOD2), microsomal hydrolase (EPHX)

Author	Paper	Type of study	Population and sample	Level of evidence GRADE	Genetic polymorphism studied	Measures of association	Results, discussion and conclusions
Berran Yucesoy Canada France 2012	Genetic Variants in Antioxidant Genes Are Associated With Diisocyanate-Induced Asthma.	Cases and controls	Workers exposed to HDI, MDI, TDI **N=353**	B	Glutathione S-transferase		This study demonstrates a statistically significant association between the studied genes associated with asthma induced by isocyanates, in both analyses, univariate and multivariate. Taken together the results of this study, we observe that the variables are significant, supporting the hypothesis that genetic variability among antioxidants contributes beside defense systems to the pathogenesis of this disease.
					SOD rs4880	P= 0.004 OR= 2.70 (1.31, 28.4)	
					GSTP1 (rs762803)	P= 0.021 OR= 6.10 (1.31-28.4)	
					*GSTM1*EPHX1 (rs2854450)*	P=0.002 OR= 7.34 (2.04, 26.5)	
					*EPHX1 (rs2740168)*EPHX1 (rs1051741)*	P=0.045 OR=8.55 (1.05, 69.9)	
					EPHX1 (rs1051741)	P=0.019 0R=10.36 (1.47, 72.96)	
					EPHX1 (rs2740171)	P=0.002 OR=6.22 (1.95, 19.82)	

Likewise, the study of genomic variants carried out by Berran Yucesoy et al. (23) finds a significant association between chronic oxidative stress of airways and the alleles SOD rs4880 and the variants EPHX1, GSTP1 associated to asthma induced by isocyanates. Otherwise the enzyme Glutathione S-hydroxylase is one of the greatest antioxidant defenses in the air-lung interface and it is essentially used as a cofactor of Glutathione S-transferase in the conjugation of hydrophilic substances, the most studied gene is GSTP1 Val105, which is highly expressed in GST in respiratory epithelium and whose variant has been reported as a protector against risk of asthma (Table 7).

Most authors agree that HLA class I molecules are highly polymorphic and therefore, there are likely candidate genes to influence in the development of a specific immune response, this situation and the small size of the analyzed samples make difficult the study of this polymorphism, B Beghé [9] observed a lack of association between this and the phenotypic presentation of the disease. FNT α A-308G is a potent cytokine involved in the inflammatory processes of airways in asthmatic people and it would be possible that this negative association resulted in a type II error, requiring studies with larger populations (Table 8).

The most effective way to detect a gene-environment interaction is measuring both genotype and environmental risk factor, this is a fundamental principle to understand an epidemiological link, D. Bernstein [24] observed that citokines, IL-4 and IL -13, play a key role in B cells and a change in IgE and it is believed that it can determine at least in part the expression of airways inflammation and allergic diseases. IL-4 may at least have an important role in the phenotype of chronic asthma associated with isocyanates (Table 8).

Jeong-Hee Choi et al. [10] suggest that natural history of asthma induced by isocyanates resembles to type I hypersensitivity responsible of allergic asthma, type specific IgE antibodies weren't detected in most of the subjects with asthma induced by isocyanates, which may be due to low understanding of isocyanate antigens and their carrier proteins, observation also suggested by Päivi Piirilä et al. [21] This finding was also confirmed by B. Beghé [9], which presuppose that HLA class I alleles and haplotypes are not associated with asthma induced by toluenes. However, HLA DQB1*0402 alleles were also studied, maybe more susceptible of specific IgE sensitization against TDI-albumin conjugates, which may contribute at least in part to the development of this disease. HLA class II polymorphism was also analyzed, observing a strong association between haplotypes DRB1*1501, DQB1*0602, DPB1*0501, which can be considered as genetic markers for the development of asthma induced by TDI. Several HLA alleles enhance specific IgE sensitization in exposed subjects. Thus, the HLA gene polymorphisms may contribute to the pathogenesis of asthma induced by toluene (Table 8).

Gene-gene interactions are of considerable importance. We must to take into account the complexity of isocyanates metabolism, as well as the immune system response, the polygenic nature of the disease and the variability of environmental exposure. Karin Broberg [25], conducted a gene-gene and shared allele connection analysis to the effect of lower airways' symptoms; (eyes, wheezing, dry cough and cough up mucus), of subjects exposed to TDI and non-exposed people. CCL5 genotype seems to have a high risk of showing symptoms associated to wheezing and cough up mucus, the risk being three times higher with the association between CCL5 and exposure to TDI. Gene-gene interactions in this study considered the complex metabolism of isocyanates as well as his immune response, gene-gene combination analysis showed no evidence of an additive effect, except for the combination GSTM1 and GSTP1 for coughing up mucus in non-exposed workers (Table 9).

Genetic variants of CTNNA3 (α-Catenin), gene that is located on chromosome 10q21.3, which are involved in the formation of tight intercellular adhesions. In the analysis conducted by D. Bernstein [26], regarding CTNNA3 (α T catenin), he found polymorphic nucleotides (rs10762058, rs7088181, rs1786929, and rs4378283) associated to occupational asthma induced by isocyanates, which suggest that CTNNA3 genetic polymorphisms may confer a risk for asthma induced by TDI increasing epithelial damage and inflammation of the airways (Table 10).

Table 8. Polymorphic relationship of HLA class I, IL4, IL5 and TNF-a A

Author	Paper	Type of study	Population and sample	Level of evidence GRADE	Genetic polymorphism studied	Measures of association	Results, discussion and conclusions
B. Beghé Italy 2004	Lack of association of HLA class I genes and TNF a-308 polymorphism in toluene diisocyanate-induced asthma.	Cases and controls	Workers exposed to TDI **N=192**	B	HLA I A (alleles) B (alleles) C (alleles) TNF-a A	p=0,03 pCc= 0,05 p= >0,05	This study shows no significant association between HLA class I genes and the polymorphism of TNF-α-308G with the risk of asthma induced by TDI. The lack of association between HLA class I genes and asthma induced by TDI can be explained by the small number of subjects studied. It is possible that the negative association with genotype TNF-α 308 G may be resulting from a type II error.
David I. Bernstein Canada USA Spain 2006	Diisocyanate asthma and gene-environment interactions with *IL4RA*, *CD-14,* and *IL-13* genes.	Cases and controls	Workers exposed to isocyanates **N=137**	B	*IL4RA* (I50V) II *IL-13* (R110Q) RR *CD14* (C159T) CT ALL THE GENES	 OR: 3.29 (1.33– 8.14) OR: 4.19 (1.35–12.68) OR: 5.2 (1.82–14.88) OR: 6.4 (1.57–26.12)	In conclusion, the data clearly show that combinations of IL4RA, polymorphisms of IL-13, and CD14 may represent genetic markers of susceptibility of asthma induced by diisocyanates HDI.

Author	Paper	Type of study	Population and sample	Level of evidence GRADE	Genetic polymorphism studied	Measures of association	Results, discussion and conclusions
Jeong-Hee Cho Korea 2009	The HLA DRB1 * 1501-DQB1 * 0602-DPB1 * 0501 Haplotype Is a Risk Factor for Toluene Diisocyanate-Induced Occupational Asthma.	Cases and controls		B	HLA C Cw*0303	p=0.005 OR 2.71 (1.37-5.35)	The HLA haplotypes DRB1 * 1501-DQB1 * 0602-DPB1 * 0501 can be considered as genetic markers for the development of asthma induced by TDI in Korean people. Various HLA alleles improve the specific IgE sensitization in subjects exposed as shown.
					BQB1 Cw*0602	p=0,028 OR 0.46 (0.22–0.92)	
					HLA DRB1*1501	P=0,008 OR 2.58(1.29–5.19)	
					DQB1*0602	p=0,06 OR 2.81 (1.36–5.79)	
					COMBINACIÓN		
					DRB1*1501-DQB1*0602-DPB1*0501	p=<0.001 OR7.23(2.32-22.51)	
					DRB1*1501-DQB1*0602	p=0.006 OR2.81(1.36-5.79)	
					DRB1*1501-DPB1*0501	p=0,001 OR: 7.8 (2.52–24.13)	
					DQB1*0602-DPB1*0501	p=<0.001 OR 7.23 (2.32–22.51)	
					ASSOCIATION ALLELE		
					IgE /Albumin-Toluene		
					A*0206	p=0.018 OR 2.39 (1.16–4.93)	
					A*0206*Cw0102	p=0.011 OR 5.18 (1.45–18.44)	
					DRB1*0802	p=0.027 OR 3.22 (1.14–9.05)	
					DQB1*0402	p=0.006 OR 4.55 (1.54–13.44)	

Table 9. Variability of phenotypic penetrance of asthma induced by isocyanates, related to the genotypes CYP1A1, CCL5, TNT α 308, Null GSTM1 GSTP1 * 105 and CCL5-403

Author	Paper	Type of study	Population and sample	Level of evidence GRADE	Genetic polymorphism studied	Measures of association	Results, discussion and conclusions
Karin Broberg Sweden 2008	Influence of genetic factors on toluene diisocyanate-related symptoms: evidence from a cross-sectional study.	Cases and controls	Workers exposed to TDI **N=246**	B	**RESPIRATORY**		By studying subjects exposed to TDI as well as non-exposed people, TDI-genes specific interactions were indicated for airway symptoms. The finding that certain genotypes modified the effect of symptoms, suggest that these symptoms are associated with each other and have common mechanisms.
					WHEEZING		
					*CYP1A1**2B	OR:14 (1.5-130)	
					CCL5 -403 Exp GA+AA	OR 3.1 (1.3-7.1)	
					TNT α 308	*p=0,043* OR 0.37(0.14-0.99)	
					DRY COUGH		
					*HLADQB1**05 Exp*	OR 2.7 (1.2-6.3)	
					TNF-308 Exp	*p=0,023* OR 0.43(0.66-7.3)	
					COUGH UP MUCUS		
					GSTM1 Exp. Nulo	OR 3.5 (1.3-9.4)	
					*GSTP1*105*	OR 3.0 (1.1-8.0)	
					CCL5-403	OR 2.9 (1.3-6.6)	

Table 10. Influence of genetic polymorphism of CTNNA3 (α-catenin), related to tissue damage and asthma induced by TDI

Author	Paper	Type of study	Population and sample	Level of evidence GRADE	Genetic polymorphism studied	Measures of association	Results, discussion and conclusions
David I. Bernstein Canada USA Spain 2012	CTNNA3 (α-Catenin) Gene Variants Are Associated With Diisocyanate Asthma: A Replication Study in a Caucasian Worker Population.	Cases and controls	Workers exposed to HDI, MDI, TDI **N= 410**	B	CTNNA3 (alpha-T catenin) (SNPs) rs7088181	p=0.01 OR 9.05 (1.6948.54)	A statistically significant association with CTNNA3 rs7088181 and rs10762058 SNPs (but not with rs4378283 and rs1786929) was observed, which were significantly associated with asthma induced by isocyanates in workers, compared with asymptomatic exposed subjects. These findings suggest that a particular genetics might reduce the expression of alpha-T-catenin, which may affect cell adhesion in the airways and may play a role in the pathogenesis of asthma induced by isocyanates.
					rs10762058	p=0.008 OR 6.82 (1.65, 28.24)	

DISCUSSION AND CONCLUSIONS

There have been few the lines of research opened on occupational asthma induced by isocyanate, we have observed that most of the papers where published between 2004 and 2013, suggesting a greater interest in the study of genetic predisposition associated to the development of diseases. We must also take into account that development of biomedical technologies for detection of nucleotide polymorphisms has been refined in recent years.

Occupational bronchial asthma induced by isocyanates is a multifactorial disease and apparently genetically heterogeneous. Regarding the findings, this disease involves a variety of genes located on different chromosomes, as well as their alleles and the substitution of nucleotides, the homozygous and heterozygous status, which may determine the phenotypic penetrance of the disease. On the other hand, we must take into account the environmental factors influencing the expression of the disease, as time and intensity of exposure to isocyanates in workplace.

Gene-gene interactions are of considerable importance. We must consider the complex metabolism of isocyanates, as well as the immune system response, the polygenic nature of the disease and the variability of environmental exposure. The finding of certain genotypes modifying the effect of symptoms, suggest that they are associated with each other and that they have common mechanisms. We observed that low circulating levels of TNF-308G and -863A may be related to the symptoms of the population exposed to TDI. An increased asthma risk was observed for carriers of the gene TNF-308G y -863A, fact coincident with the studies conducted by A. Balboni [7] y C.E. Mapp et al. [8] that may hypothetically contribute to formation of powerful reactive metabolites by the oxyacetylation in airways, which would increase the individual risk of symptoms associated to TDI.

The presence of inflammation of airways is a relevant biochemical characteristic of asthma. Oxidative stress, with the formation of oxygen free radicals, is a key component in inflammation, suggesting that polymorphic GST, specially the mu class, play an important role in modulating the individual's response to the occupational exposure to diisocyanates, a fact that was reported by Päivi Piirilä [21]. The analysis of IgE specific antibodies was observed only in 10-30% of the cases suggesting that other immunological or non-immunological mechanisms are involved in the etiology of this disease.

The risk of developing asthma increase with the time of exposure, but the time course follows a pattern of an epidemic curve, with an average period of 1-2 years, compatible with the development of sensitization and asthma in a susceptible population. However, the latent period between the start of exposure and the start of symptoms is quite variable, Cristina E et. al [22], studied the gene GSTP1*Val105 suggesting that it confers protection against asthma induced by TDI, being protective against the disease or at least a modulator, so that some subjects develop asthma after several years of exposure and it would partly explain why some exposed subjects develop asthma and others not.

The strong role of NAT, observed by Harriet W. et al. [13], may be due to formation of immunological haptens coming from isocyanate, the more favorable formation taking place in slow acetylators than in rapid acetylators, these results suggest that, in addition to GST, NAT play an important role in the modulation of the individual susceptibility of asthma induced by diisocyanate exposure.

HLA associations don't always provide clear evidence of a specific immunological response in asthma caused by low molecular weight sensitizers. In the study of B. Beghé [9] she demonstrated that FNT α A-308G polymorphism doesn't play the major role in the disease.

The most studied gene is GSTP1 Val105, which is strongly expressed in GST in respiratory epithelium and whose variant has been reported as protective against risk of

asthma; Berran Yucesoy et al.[23], however, this study is inconsistent when dealing with DA, unlike the finding of the study of Cristina E.[22] who does find a protective association and modulator of asthma associated with isocyanates.

Genetically determined reduced expression of α T catenins and their influence at the level of cell adhesions in the airways may play a role in the pathogenesis of this occupational disease. Further studies will be needed to make clear the exact mechanism by which CTNNA3 contributes to asthma induced by TDI[26]. Another finding of D. Bernstein[24] suggest that IL-4 and IL-5 are expressed in the bronchial tissues 48 hours after inhalation of isocyanate, only IL-5 persist in patients with chronic asthma after elimination of TDI from workplace. Concluding that data clearly show that combinations of IL4RA, polymorphisms of IL-13, and CD14 may represent genetic markers of susceptibility to asthma induced by diisocyanates specially by HDI.

The most effective way for detecting a gene-environment interaction is to measure both the genotype and the environmental risk factor, this principle is fundamental to understanding an epidemiological link. The phenotype represents a genotype-environment interaction, because the disease will appear only if the exposure exists and phenotype is not expressed if it is not genetically determined (susceptibility). There is also the problem of incomplete penetrance, which means that an individual has a predisponent polymorphism and doesn't develop the disease, as opposed to one who doesn't possess it, and he does develop it if the exposure is sufficiently intense (phenocopy). Genetic susceptibility can influence the disease in different ways. First, on the risk of disease itself; second, it can increase by environmental exposure to isocyanate, and third, the risk factor can increase the genetic effect[12].

Genetics has a significant influence in occupational asthma induced by isocyanates, but the 'weight' of genetic susceptibility and of the gene-environment interaction have not been fully elucidated yet. To fully understand the genetics of this complex disease, it is essential to establish a multidisciplinary team to identify metabolic pathways and define the precise genotype of the disease. Once we have understood the mechanisms and how genetic polymorphism interacts with environmental factors, we must identify subjects with a predisposition to develop occupational asthma or other allergic diseases. These questions remain open, since, so far, the search for genes in asthma and allergies continues, as much of the information is still fragmentary.

The epidemiological approach makes it possible to evaluate models to investigate the relationship between genetic susceptibility and environmental risk factors. Understanding the relationships between genes and working environment has implications in society and for workers' health, given that some environmental factors and from workplace that influence the genetic risk can be modified.

ACKNOWLEDGEMENTS

The authors acknowledge Dr. Jerome B. Maqueda for reviewing this document. The authors also acknowledge the Institute of Health Carlos III. National Institute for Safety and Hygiene at Work. Madrid. Spain.

REFERENCES

1. Flaviis MI de. Asma ocupacional por exposición a isocianatos, mezclados con otras sustancias y factores asociados. [Tesis doctoral]. Universidad Centro Occidental Lisandro Alvarado. Venezuela, Barquisimeto. 2008.
2. Orriols, R, Drobnic, ME, Muñoz, X. et al. Asma ocupacional por isocianatos: estudio de 21 pacientes. *Med Clin (Barc).*1999; (113):659-662.
3. Ministerio de Empleo y Seguridad Social de España [sede web].[actualizado 15 de Diciembre 2013] CEPRROSS; [1 pantalla]. Disponible en: Internet_1/Estadistica/Est/Observatorio_de_las_Enfermedades_Profesionales/cepross2k11/Partes_comunicados/index.htm.

4. Cocker J, Biological monitoring for isocyanates. Ann Occup Hyg. 2011; 55 (2):127-31.

5. Shams KA, Boldú J, Tiberio G, et al. Registro de enfermedades respiratorias de origen laboral en Navarra. An. Sist. Sanit. Navar. 2005; 28 (1): 135-143.

6. Ministerio de Seguridad y Empleo. Catálogo de publicaciones del INSHT. Límites de exposición profesional para agentes químicos en España 2013. Disponible en: http://wwwinsht.es/catalogopublicaciones/. ISBN: 078-84-7425-808-0.

7. Balboni A, Baricordi OR, Fabbri LM, Gandini E, Ciaccia A, Mapp CE. Association between toluene diisocyanate-induced asthma and DQB1 markers: a possible role for aspartic acid at position 57. Eur Respir J, 1996, 9:207–210.

8. Mapp C E, Begheá B, Balboni A, Zamorani G, et. al. Association between HLA genes and susceptibility to toluene diisocyanate-induced asthma. Clinical and Experimental Allergy. 2000; 30: 651-656.

9. Beghé B, Padoan M, Moss, CT, Barton SJ, et. al. Lack of association of HLA class I genes and TNF a-308 polymorphism in toluene diisocyanate-induced asthma. Allergy. 2004; 59 61–64.

10. Choi JH, Lee KW, Kim CW, Park CS, Lee HY, Hur GY, et. al. The HLA DRB1*1501-DQB1* 0602-DPB1*0501 Haplotype Is a Risk Factor for Toluene Diisocyanate-Induced Occupational Asthma. Int Arch Allergy Immunol 2009; 150:156-163.

11. Valentino M, Rapisarda V. Evoluzione dell'asma da isocianati in rapporto alla cessazione dell'esposizione: studio longitudinale su 50 soggettiClinica di Medicina del Lavoro, Università di Ancona - Polo Didattico Torrette. G Ital Med Lav Erg. 2002; 24 (1) 26-31.

12. C.E. Mapp. O.Vandenplas y J.-L. Malo. El papel de los factores genéticos en el asma ocupacional. SERIE "CONTROVERSIAS EN EL ASMA OCUPACIONAL". Eur Respir J (edición española). 2004; 5 (1): 29-35.

13. Harriet W, Piirilä P, Christina, Ritva L, et. al. N-Acetyltransferase genotypes as modifiers of diisocyanate exposure-associated asthma risk. Pharmacogenetics. 2002; 12: 227-233.

14. Karimi A, Shokouhi Shoormasti R, Miri S, et al. Asthma Symptoms and Specific IgE Levels among Toluene Diisocyanate (TDI) Exposed Workers in Tehran, Iran. Iran J Public Health. 2013 1; 42 (4):397-401.

15. Flack SL, Fent KW, Trelles Gaines LG, Thomasen JM, et. al. Quantitative plasma biomarker analysis in HDI exposure assessment. Ann Occup Hyg. 2010 Jan. 54 (1):41-54.

16. Sabbioni G, Gu Q, Vanimireddy LR. Determination of isocyanate specific albumin-adducts in workers exposed to toluene diisocyanates. Epub 2012 Jan;17 (2):150-9.

17. Kakooei H, Shahtaheri SJ, Karbasi HA. Evaluation of workers' exposure to methylene diphenyl diisocyanate (MDI) in an automobile manufacturing company, Iran. Int J Occup Saf Ergon. 2006; 12 (4):443-9.

18. Thomasen JM, Nylander-French LA. Penetration patterns of monomeric and polymeric 1,6-hexamethylene diisocyanate monomer in human skin..J Environ Monit. 2012 Feb. 14 (3):951-60.

19. Arrandale V, Meijster T, Pronk A, Doekes G, et. al. Skin symptoms in bakery and auto body shop workers: associations with exposure and respiratory symptoms. Int Arch Occup Environ Health. 2013 Feb; 86 (2):167-75.

20. Balboni A, Baricordi OR, Fabbri LM, Gandini E, Ciaccia A, Mapp CE. Association between toluene diisocyanate-induced asthma and DQB1 markers: a possible role for aspartic acid at position 57. Eur Respir J, 1996, 9:207–210.

21. Piirilä P, Wikmanb H, Luukkonenc R, Kääriä K, Rosenbergb C, Nordmana H. Glutathione S-transferase genotypes and allergic responses to diisocyanate exposure. Pharmacogenetics 2001; 11:437-445.

22. Mapp CE, Fryer AA, De Marzo N, Pozzato V, Padoan M, Boschetto P, et.al. Glutathione S-transferase GSTP1 is a susceptibility gene for occupational asthma induced by isocyanates. J Allergy Clin Immunol 2002; 109:867-72.

23. Yucesoy B, Victor J Johnson, Lummus Z.l, Kissling G.E, et. al. Genetic Variants in Antioxidant Genes Are Associated With Diisocyanate-Induced Asthma. Toxicological sciences. 2012; 129 (1):166–173.

24. David I. Bernstein, Ning W, Campo P, Ranajit C et. al Diisocyanate asthma and gene-environment interactions with *IL4RA*, *CD-14,* and *IL-13* genes. Ann Allergy Asthma Immunol. 2006; 97: 800-806.

25. Broberg K, Tinnerberg H, Axmon A, Warholm M, Rannug A, Littorin M. Influence of genetic factors on toluene diisocyanate-related symptoms: evidence from a cross-sectional study Environ Health. 2008; 7: 15.

26. Bernstein D I, Kashon M, Lummus Z L, Johnson VJ, Fluharty K, Gautrin D, et.al. CTNNA3 (α-Catenin) Gene Variants Are Associated With Diisocyanate Asthma: A Replication Study in a Caucasian Worker Population. Toxicological Sciences 2013;131(1): 242-246.

27. Creely KS, Hughson GW, Cocker J, Jones K. Assessing isocyanate exposures in polyurethane industry sectors using biological and air monitoring methods. Institute of Occupational Medicine. Ann Occup Hyg. 2006. 50 (6):609-21.

28. Martínez GC, Rego FG. Enfermedades respiratorias de origen ocupacional. Arch Bronconeumol. 2000; 36 (11):631-44.

29. Lemons AR, Bledsoe TA, Siegel PD, Beezhold DH, Green BJ. Development of sandwich ELISAs for the detection of aromatic diisocyanate adducts. J Immunol Methods. 2013Nov; 39 (1-2): 66-70.

30. Pearson RL, Logan PW, Kore AM, Strom CM, Brosseau LM, et. Al. Isocyanate exposure assessment combining industrial hygiene methods with biomonitoring for end users of orthopedic casting products. Ann Occup Hyg. 2013; 57 (6):758-65.

31. Tinnerberg H, Broberg K, Lindh CH, Jönsson BA. Biomarkers of exposure in Monday morning urine samples as a long-term measure of exposure to aromatic diisocyanates. Int Arch Occup Environ Health. 2013.

32. Schroeter JD, Kimbell JS, Asgharian B, Tewksbury EW, et. al. Inhalation dosimetry of hexamethylene diisocyanate vapor in the rat and human respiratory tracts. Inhal Toxicol. 2013 Feb; 25 (3):168-77.

33. Jones K, Cocker J, Piney M. Isocyanate exposure control in motor vehicle paint spraying: evidence from biological monitoring. Ann Occup Hyg. 2013 Mar; 57 (2):200-9.

34. Wisnewski AV, Redlich CA. Recent developments in diisocyanate asthma. Curr Opin Allergy Clin Immunol. 2001 Apr; 1 (2):169-75.

35. Bello D, Woskie SR, Streicher RP, Liu Y, Stowe MH, Eisen EA, et. al. Polyisocyanates in occupational environments: a critical review of exposure limits and metrics. Am J Ind Med. 2004 Nov; 46 (5):480-91.

36. Nakashima K, Takeshita T, Morimoto K. Diagnosis and prevention of diseases induced by isocyanate. Environ Health Prev Med. 2002 May; 7 (2):40-6.

37. Sabbioni G, Dongari N, Kumar A. Determination of a new biomarker in subjects exposed to 4,4'-methylenediphenyl diisocyanate. Biomarkers. 2010 Sep; 15 (6):508-15.

38. Tarlo SM, Liss GM. Prevention of occupational asthma--practical implications for occupational physicians. Occup Med (Lond). 2005 Dec; (8):588-94.

39. Berode M, Jost M, Ruegger M, Savolainen H. Host factors in occupational diisocyanate asthma: a Swiss longitudinal study. Int Arch Occup Environ Health. 2005 Mar; 78 (2):158-63.

40. Williams NR, Jones K, Cocker J. Biological monitoring to assess exposure from use of isocyanates in motor vehicle repair. Occup Environ Med. 1999 Sep; 56 (9):598-601.

41. C Martínez González, G Rego Fernández. Enfermedades respiratorias de origen ocupacional. Arch Bronconeumol. 2000; 11 (36) 631-44.