Adapting the ELEAT (Early Life Exposure Assessment Tool) to Portugal – a pilot study to tackle gene-environment interactions in Autism Spectrum Disorder

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ABSTRACT

Background: Autism Spectrum Disorder (ASD) is a pervasive and clinically heterogeneous neurodevelopmental disorder by deficits in social communication and interactions skills, and repetitive and stereotyped behaviours. It has becomes apparent that ASD has a strong genetics component, however, the level of heritability is still debated, suggesting that modifiable non-genetic factors may play an important role in ASD. Pre-, peri and post-natal exposure to a variety of environmental factors have previously been implicated in its etiology. However, the comprehensive assessment of environmental exposure in ASD in large datasets from variable environmental settings is still lacking.

Methods: To examine the role of environmental exposures in a population of Portuguese children with ASD, we translated, adapted and are piloting the Early Life Exposure Assessment Tool (ELEAT). The ELEAT was originally developed at the University of California Davis to assess environmental factors in studies of neurodevelopmental disorders. It is a questionnaire filled by mothers of children with ASD, enquiring about Demographic Information, Maternal Conditions/ Medical Interventions, Breastfeeding and Child Diet, Maternal Diet, Supplements, Lifestyle, Home and Environment, Environment, Occupation and Exposures. The ELEAT gathers information about

environmental exposure along key phases for early neurodevelopment, including 3 months prior to conception, pregnancy, labor and delivery and the first year of life of the child.

Results: The large majority of mothers were sure about their answers for all modules, with some clear difficulties reported only for the module Occupations/Exposures for a small fraction of the group. Most of the mothers considered the ELEAT to be a little too long, but generally found that the instructions were clear and, most importantly, agreed that the questions were important.

Conclusions: Integration of the pilot feedback will allow us to enhance the tool and optimize its usage in Portuguese-speaking communities, improving its capacity to assemble accurate environmental data from diverse cultural settings, and to be extended to larger population datasets. Combined with genetic and clinical data, the ELEAT will contribute to the identification of modifiable lifestyle and environmental risk factors for ASD. Such evidence may eventually provide the opportunity for disease prevention or reduced severity by mitigating exposure when genetic susceptibility is identified early in life.

Keywords: Autism spectrum disorder, gene-environment interactions

INTRODUCTION

Autism spectrum disorder (ASD) is a complex neurodevelopmental disorder that manifests in early childhood. It is characterized by impairments in communication and social behavior, as well as by repetitive behaviors (American Psychiatric Association, 2013). ASD encloses a group of heterogeneous neurodevelopmental syndromes and occurs in all social, racial, ethnic and socioeconomic groups, with a prevalence of about 5 out of 1000 children in Portugal (nosso estudo?). In terms of global prevalence, studies in different countries estimate that the median ASD prevalence is that of 17/10 000, widely varying among countries (being smaller in Europe when compared to USA) (Elsabbagh et al., 2012), but which have been markedly rising for the past 30 years (Lai, Lombardo, & Baron-Cohen, 2014; Elsabbagh et al., 2012). There has been much discussion about the nature of the increase in prevalence of ASD, and recent research paint a picture of apprehensiveness. But what makes it so difficult to understanding these prevalence? Why do we keep searching for alternative explanations?

In the last few decades, autism research has grown exponentially, however some fundamental questions remain unanswered, namely, we do not yet understand its causes. The etiology of autism is complex and poorly understood due to the broad variability or clinical

heterogeneity of ASD. We know that ASD has been demonstrated to be highly heritable (Sandi, Lichtenstein, Kuja-Halkola, Hultman,Larsson & Reichenberg, 2017). Multiple genes have been implicated in autism, explaining approximately 10% to 15% of cases autism spectrum disorders (Abrahams & Geschwind, 2010; Ozonoff, Young, Carter, et al., 2011) as well as several genetic syndromes that are known to have significant associations with ASD. But these explain only a minority of cases (Devlin & Scherer, 2012; Zafeiriou, Ververi, Dafoulis, Kalyva & Vargiami, 2013).

Indeed, there is more to the story than can be solely accounted by genetics. That is, although genetic factors are clearly important, and might be largely responsible for the occurrence of autism, they cannot fully explain all cases. Therefore, it is likely that in addition to a certain combination of autism-related genes, specific non-genetic environment modulators might act as risk causes, triggering the development of autism, perhaps acting in concert with genetic susceptibilities (Hallmayer, Cleveland, Torres, Phillips, Cohen, Torigoe, ... & Lotspeich, 2011; Grabrucker, 2013).

Since there has been an accumulation of evidence pointing to the role played by environmental factors in ASD risk, a considerable number of studies have focused on this area, which was previously underexplored. The developing human brain is understood to be exquisitely susceptible to injury caused by toxic chemicals in the environment. This vulnerability is greatest during embryonic and fetal life, and may be especially great in the first trimester of pregnancy. There exists windows of susceptibility in early development that have no counterpart in the mature brain (Rodier, 1995; Rice & Barone, 2000). Furthermore, when mechanisms responsible for body homeostasis are debilitated, the interaction with potentially hazardous environmental factors may result in internal dysregulation such as abnormal immune activation, increased oxidative stress, as well as genomic instability. It has been established that immune system dysregulation, including infections in early pregnancy, prenatal maternal inflammation, and increased neuronal inflammation, could be contributing factors for the development of ASD (Koufaris & Sismani, 2015).

Evidence about overall environmental risk factors of ASD is growing. Studies have also shown that environmental factors have the ability to affect epigenics. These are mechanisms that biochemically modify DNA or histones affecting gene expression, without changing the DNA sequence (Modabbernia, Velthorst, & Reichenberg, 2017). Environmental factors associated with ASD that can affect these mechanisms include exposure to heavy metals, low levels of dietary folate (Koufaris & Sismani, 2015), as well as maternal use of valproate, used primarily to treat epilepsy and bipolar disorders (Kalkbrenner, Schmidt & Penlesky, 2014), due to its ability to inhibit histone deacetylase and interference with folic acid metabolism (Modabbernia et al., 2017). Air

pollution has also been associated with ASD, a study found elevated risk for ASD in adjusted analyses of the top quartile of exposure to chlorinated solvents, heavy metals, diesel particles and other individual compounds (Lyall, Schmidt, & Hertz-Picciotto, 2014)

Seeing as ASD has an early onset development , it comes as no surprise that a number of studies have been focused on maternal lifestyle, with attempts to correlate prenatal factors to ASD development. A study found that by every 10 year increase in maternal and paternal age, the increase of ASD risk in the offspring is that of 18% and 21% respectively. In addition to this, it has also been hypothesized that the increase in ASD risk may be attributed to the accumulation of toxins, such as persistent organic pollutants (PCBs) and EDCs, in the body since it is probable that older parents lived in times prior to chemicals bans and experienced more years of accumulation (Kalkbrenner et al., 2014).

A efficient way to measure the relationship between ASD and environmental exposures is, ideally, through a large case-control study, which demands objective information and a direct measure of compounds or metabolites in bio-specimens. However, in Portugal, this information and archived samples are not often accessible for use in case-control and dataset cohort studies. Additionally , this type of prospective studies are commonly complex, take a long period and require a substantial funding, which may lead to incomplete data and biological sample collection, as well as parents dropping out of the study, which consequently leads to the loss of records associated, for example, to medical conditions, events and interventions, including pharmaceutical exposure.

In this sense, the inclusive assessment of environmental exposure in ASD in large datasets from variable environmental settings was lacking. The Early Life Exposure Assessment Tool (ELEAT) was originally developed in California by researchers of University of California Davis, it is a questionnaire that aims to assess environmental exposures that have been associated with ASD development. It allows for an indirect approach given that it uses measurements of exposures in specific microenvironments (e.g. home) and human activity pattern (e.g. maternal lifestyle) data to predict levels of fetal/infant exposure. Indirect exposures, such as this one, are used frequently on account that they require less resources than the assessment of direct exposures.

The aim of this study was to pilot a Portuguese version of the Early Life Exposure Assessment Tool (ELEAT) for the assessment of the role of environmental exposures in a population of Portuguese children with ASD.

METHOD

Participants

The first group was composed of 20 mothers of children with ASD recruited from the Autism Spectrum Disorder in the European Union (ASDEU) project. This project aimed to investigate the prevalence of autism in 12 countries in the European Union, including Portugal, which was finalized in 2017. The second group was integrated by 12 mother workers employed by Instituto Nacional de Saúde Ricardo Jorge (INSA). These mothers self-administered the ELEAT survey on paper prior to the focus group session.

From all the mothers who agreed to participate, all attended the focus group.

Material

The ELEAT's objective is to understand how environmental risk factors can influence ASD development.

This tool is divided in 10 modules: Demographic Information, Maternal Conditions/Medical Interventions, Breastfeeding and Child Diet, Maternal Diet, Supplements, Lifestyle, Home and Environment, Environment, and Occupation and Exposures, and Instrument Evaluation. This questionnaire is constituted by 259 items (some may be skipped with gateway questions), corresponding to 80 pages, which intend to asses 3 different periods: it examines maternal exposure to exogenous factors from 3 months before conception to the first year of the child's life. Therefore, covering crucial phases of early neurodevelopment, with each module analyzing different sources of exposure.

In addition to the paper version, an online version was created in the RedCAP survey, a secure web application for building and managing online surveys and databases. Here the mothers were able to access the questionnaire through a link, and were given the option to save their answers and proceed filling it in later. This became the main filling method not only because it was more organized, but mainly because, seeing as the questionnaire is extensive, the fact that the mothers could save their answers and return to it later made it easier and more appealing due to busy lifestyles. However, the mothers had always the chance to fill out the questionnaire in paper version.

Procedure

Mothers of children with ASD and typically-developing children filled a Portuguese ELEAT, which was translated from the UK version due to the greater similarities in lifestyle and culture compared to the original US version. For example, the American ELEAT included questions regarding fish

types that were not available in Portugal, as well as housing typologies. Cross-cultural problems that might compromise its validity are minimized by European similarities regarding lifestyle, habits, dietary, household and other daily items, comparable access to healthcare and education, as well as employment and infrastructure, and no major cultural adaptations were made.

After being translated, the ELEAT was piloted in a group of mothers of typically-developing and mothers of ASD children. It was completed mostly completed online, although a paper version was also available, with an average completion time of 60 minutes.

Focus Groups were conducted by the same psychologist with experience in exploring health and environmental themes. Both focus groups were supported by specific scripts, which was addressed to improvement of questions and modules, determine the most logical question order, simplify formatting, and shorten the instrument.

Simultaneously, another research member took notes of salient patterns, answers, suggestions and themes from the discussions.

Both focus group sessions were recorded, and took around 1 hour and half.

RESULTS

Two focus groups were constituted to obtain a broad perspective with respect to Socio-demographic characteristics of the sample (Table 1). Within each focal group the characteristics of the mothers were homogeneous, however, between both focus groups these were more heterogeneous, especially regarding the group with mothers of children with ASD. All mothers were at least 18 years of age, with an averaged 40 years of age, although mothers with ASD children, in Group 1, had a broader range age. Mothers had one or more children. Typically-developing children and ASD children's age ranged from 2 to 12 years. Most mothers, in both groups, were born in Portugal and spoke Portuguese fluently at home (75% vs. 100% respectively). All mothers with typically-developing children and 45% of mothers of children with ASD had a university degree.

Table 1 - Socio-demographic characteristics of the sample

	Children with ASD	Typically-developing children
Child age mean (age range)	9.3 (8-12)	5.0 (2-10)
Mother age mean (age range)	39.6 (32-50)	40.5 (36-45)
Born in Portugal %	75	100
Resident in Portugal %	100	100
Education		
Basic %	55	8
University degree %	45	92

The focus group discussion was productive and generated very good thoughts that produced important adaptations for the improvement of the ELEAT. Despite the heterogeneity between groups, identical opinions and suggestions arose.

Concerning length, in general, all mothers agreed that the questionnaire was too long and should be shortened. 34% of the mothers considered the ELEAT very long, and 56% considered it slightly long (Figure 1).

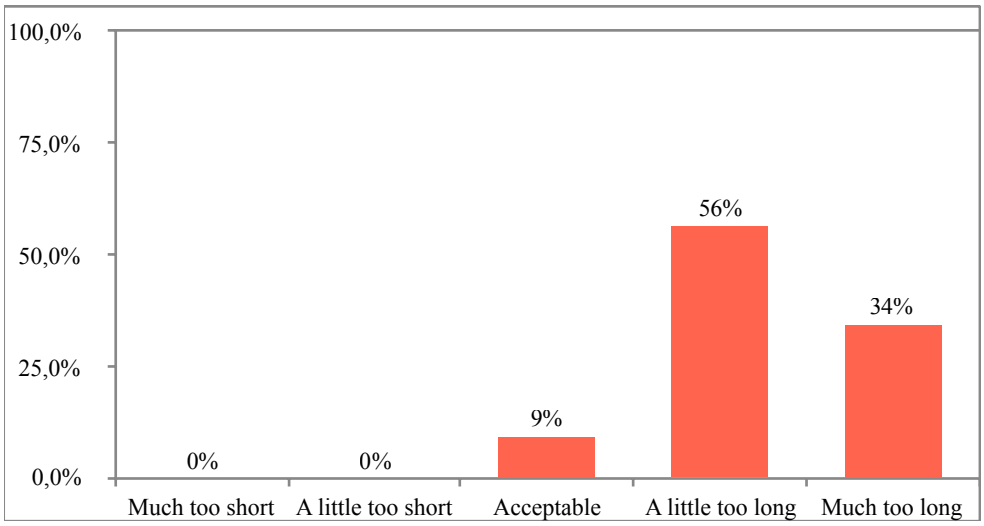

Figure 1 - Overall questionnaire length rating (% answers)

However by the end of the discussions these same women felt that it would be best to keep as many questions as possible to avoid omitting potentially important exposure information**.** In a Lickert-type scale, 63% of mothers agreed and 20% of mothers somewhat agreed that the questions were important, but 10% were neutral (Figure 2).

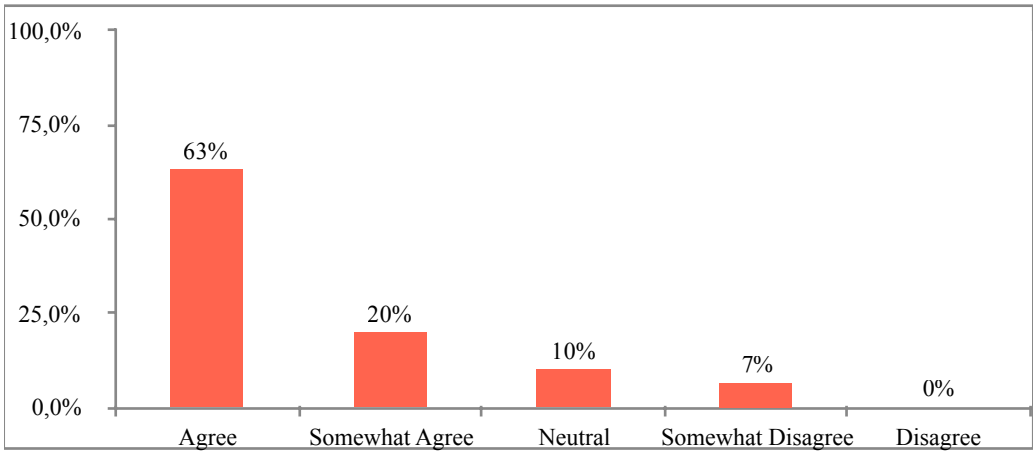

Figure 2 - Percentages of importance of questionnaire questions

As Figure 3 shows, 48% agreed and 45% slightly agreed that they were sure about their answers. In fact, one of the concerns discussed and addressed was the certainty level of answer seeing as some mothers gave birth many years ago, and the questionnaire is very detailed and requires them to recall details during their pregnancy. However, the strategy used by mothers was to think about their daily habits, considering that these were not significantly altered during pregnancy.

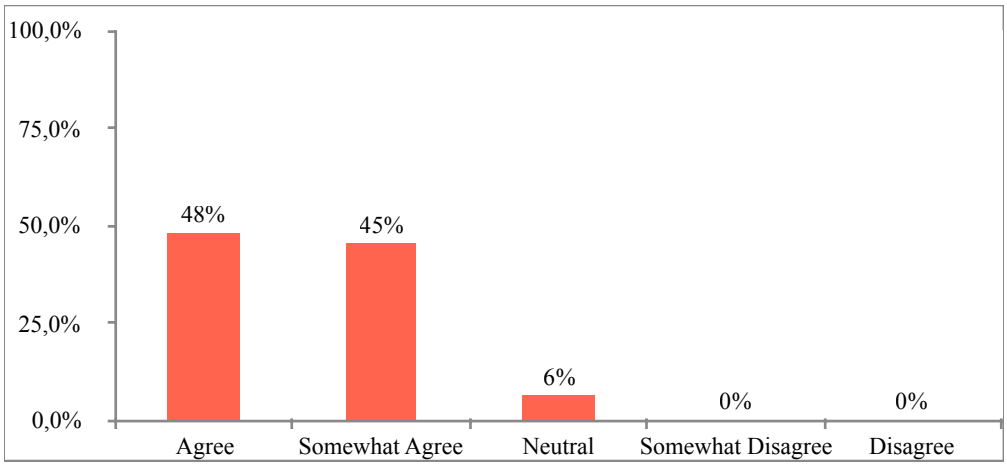

Figure 3 - Certainty regarding answers for the different modules (% answers)

In all modules, mothers reported above 50% of high confidence about their answers. In four modules (Maternal Condition/Medical History, Breastfeeding, Maternal Diet and Home Environment) between 70% and 88% of the mothers reported high certainty concerning their given answers. Only in Occupation and Exposures did the mothers express lower confidence, with about 52% reporting high certainty (Figure 4).

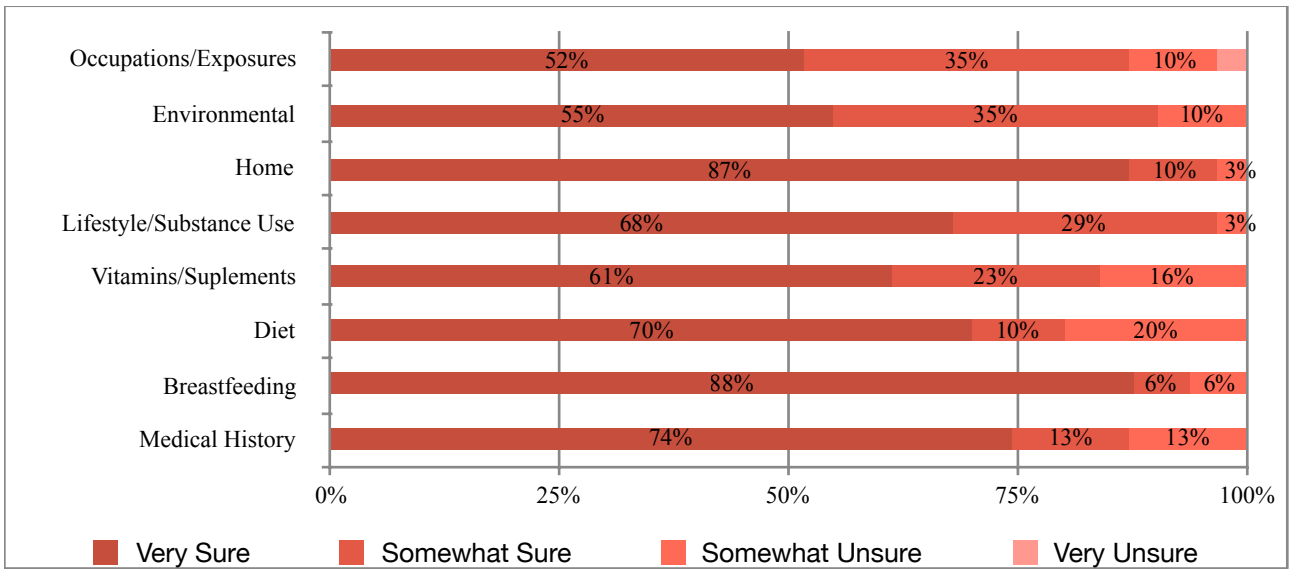

Figure 4 - Certainty regarding answers for the different modules (% answers)

In fact, the difficulty to recall detailed information from pregnancies was dependent on the subject matter and can be dependent on the interval of time between the pregnancy events and survey administration.In general, similar concerns were reported in both group discussions regarding the length, detailed and complexity of the questionnaire. With the main positive conclusions were that the questions were direct and well written, 68% of the mothers agreed and 23% slightly agreed that the instructions were clear. However, mothers suggested that vocabulary and medical vocabulary should be replaced with simpler words and phraseology to broaden survey use for individuals with limited language proficiency.

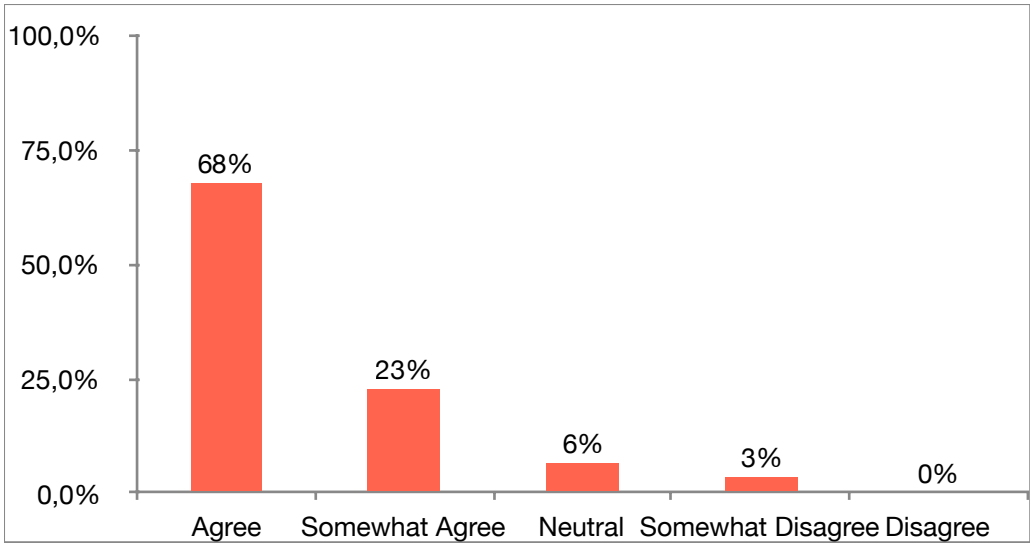

Figure 5 - Percentages of clarity about questionnaire questions

In general, all mothers, favored moving the questionnaire from paper version to online administration, as well as suggesting that the administration should be assisted, thus allowing a more efficient and easier completion. Mothers also favored the flexibility provided by online administration, allowing them to complete the survey in multiple sessions as their schedules allowed.

In addition, another very relevant result was that no question scored 20% regarding to “Do not know” answer. In fact, only 3 questions scored 19% on the "Do not know" answer and these questions belonged to the supplements module (for example: Vitamin B6?, Vitamin B12?, Calcium?)

DISCUSSION

The goal of this study was to pilot a Portuguese version of the Early Life Exposure Assessment Tool (ELEAT) for the assessment of environmental exposures roles in a population of Portuguese children with ASD, by conducting two focus groups in order to generate a discussion focused on question clarity, content, module length, formatting, and mode of administration.

First and foremost, we think that both focus group discussions were fundamental to improve and adapt the usability and contents of the ELEAT. In both groups, all mothers participated actively and yielded important suggestions, adaptions, thoughts, ideas, experiences and advices. In general, this pilot study provided us a great evidence for considering that all content discussed on the focus groups were crucial and indispensable for the update of both structural reviews and some words and details of ELEAT, becoming it less dubious and more accessible to broader socio-economic levels of the population.

Morgan (1997) defined focus groups as a research technique that collects data through group interactions about a particular topic suggested by the researcher. It can also be characterized as a resource to understand the process of constructing the perceptions, attitudes and social representations of groups of human beings (Smith, 2000).

The starting point for conducting a research project, which is supported by the use of focus groups, is the clarity of its purpose. Methodological decisions depend on the objectives established. This will influence the composition of the groups, the number of elements, the homogeneity or heterogeneity of the participants (culture, age, gender, social status, etc.). All of these factors can influence the discussion process and the finals results. We think that our results were neither influenced nor limited by these factors. Although mothers were not randomly selected from the

population, our sample size was relatively small and each group was relatively homogeneous regarding to socio-demographic characteristics, we think that, overall, mothers gave us a variety of thoughts, suggestions, ideas and doubts. And most importantly, these perspectives were similar between groups.

The ELEAT was upgraded in response to focus group considerations. On the one hand, clinical content was reduced and we think that this could minimize the redundancy and increased user-friendliness in order to mother can self-administered the ELEAT. In addition, this simplification may decrease the literacy level required, and consequently improve data collection accuracy for those with less education. It is very important that the ELEAT can be sensitive to cultural factors and embrace diversity and foster an atmosphere in which the varied life experiences of parents are recognized and respected. Before this pilot study, the ELEAT had already undergone a pilot study in the United States and England. Our pilot study also allows for the further improvement of this instrument prior to making the survey available for global use.

On the other hand, results also showed that mothers felt that the instructions to answer the tool were clear, and the majority felt sure about their answers. In addition to this, they also appreciated the possibility of filling it online, and gave very positive feedback regarding this process. Feedback from this module is essential, considering that this tool is still being molded to improve its capacity to assemble accurate data from diverse populations. Due to the use of scientific terms in the questionnaire, it is especially important to offer assistance to participants with lower education in order to guarantee accurate data collection. By tackling this and the length of the questionnaire, it is plausible that the selection of “don’t know” will decrease.

Therefore, ELEAT’s ability to measure multiple factors simultaneously gives it an advantage over studies that measure single exposures. It is important to note that this tool aims simply to gather information about environmental exposure and is not to be used as a diagnosis instrument. It is also important to note that sole exposure to such environmental factors is not sufficient to set in motion the mechanisms that lead to neurodevelopmental problems. It is however the combination of exposure and genetic susceptibility to such factors that may lead to heavier consequences.

Taking into account that this is a standardized tool, the ELEAT has the potential of being applied in different countries and gather a large amount of data that could be analyzed together and lead to meaningful findings. It has been noted that the translation of instruments such as the ELEAT may present some cross-cultural problems, thus compromising its validity. However, in this pilot study, these are minimized by the fact that there is not a large discrepancy between the lifestyle and habits of British vs Portuguese mothers.

The main limitation of this tool was its length. As seen by the results section, this tool was considered to be extensive. This could be one of the principal reasons that could limite the sample size of futures studies, once mothers have to be motivated to fill out the entire ELEAT. In addition to this, the questions have proven themselves complex to extract quantitative information from, which is necessary for the creation of a score. Therefore, the creation of a rational to transform qualitative into quantitative results is a crucial step to improve the statistical power of ELEAT conclusions.

This pilot study was an important step for future studies that intend to use the ELEAT to identify environmental risk factors for ASD. In fact, there has been an accumulation of evidence pointing to the role played by environmental factors in ASD risk, and, given the difficulty of obtaining information on the environmental exposure, ELEAT can assume a very important role in case–control studies, once it is important to have a questionnaire based on the opinion of the mothers of who fills it. Currently, most of studies that focus on the role of environmental exposures during fetal development and early postnatal time periods depends on surveys administered to mothers about these early periods. With the exception of some eventual exposures registers in existing records (such as medical files, air pollutant and water monitoring), case–control studies have few data other than parental descriptions, once many human exposures depend on individual behaviors and cannot be retrospectively determined from existing records or exposure databases.

Additionally, identification of early biological markers requires a specimen collection in firs years. Cohort studies fill all these gaps, and the ELEAT study can therefore provide an excellent tool for prospectively assessing environmental risk and protective factors, collecting biological markers that may correspond to early signs of ASD.

The addition of genetic factors has proven to be complementary to the questionnaire and therefore allow for more meaningful results. This combination helps reach more solid conclusions considering the fact that it provides a biological explanation to why the potential factors found by the ELEAT may be associated with ASD risk. This combination helps reach more solid conclusions considering the fact that it provides a biological explanation to why the potential factors found by the ELEAT may be associated with ASD risk and we thing that ELEAT and other cohort studies are essential to progress in understanding ASD etiology.

CONCLUSIONS

The main positive conclusions were that the questions were generally considered clear and important, and that most mothers did not have major difficulties recalling details and timing of exposures occurring in their children's early life. Some concerns were however reported regarding the length and complexity of the questionnaire.

Integration of the pilot feedback allows us to enhance the tool for its application in Portuguese-speaking communities, improving its capacity to assemble accurate environmental data from diverse cultural settings, and to be extended to larger population datasets. Combined with genetic and clinical data, the ELEAT will contribute to the identification of modifiable lifestyle and environmental risk factors for ASD. Such evidence may eventually provide the opportunity for disease prevention or reduced severity by mitigating exposure when genetic susceptibility is identified early in life.

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