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European Medical Education Initiative on Noonan syndrome: A clinical practice survey assessing the diagnosis and clinical management of individuals with Noonan syndrome across Europe

Sixto García-Miñaúr [a,*], Emma Burkitt-Wright [b], Alain Verloes [c], Guftar Shaikh [d], Jan Lebl [e], Ingegerd Östman-Smith [f], Cordula M. Wolf [g,h], Eduardo Ortega Castelló [i], Marco Tartaglia [j], Martin Zenker [k], Thomas Edouard [l]

[a] Institute of Medical and Molecular Genetics (INGEMM), Hospital Universitario La Paz Research Institute (IdiPAZ), Hospital Universitario La Paz, Madrid, Spain
[b] Manchester Centre for Genomic Medicine, Manchester University NHS Foundation Trust and University of Manchester, Manchester, UK
[c] Department of Genetics, APHP-Robert Debré University Hospital and Université de Paris Medical School, Paris, France
[d] Department of Paediatric Endocrinology, Royal Hospital for Children, Glasgow, United Kingdom
[e] Department of Pediatrics, 2nd Faculty of Medicine, Charles University and University Hospital Motol, Prague, Czech Republic
[f] Department of Pediatrics, Institute of Clinical Sciences, Sahlgrenska Academy, Gothenburg University, Gothenburg, Sweden
[g] Department of Congenital Heart Defects and Pediatric Cardiology, German Heart Center Munich, Technical University of Munich, Munich, Germany
[h] DZHK (German Centre for Cardiovascular Research), Partner Site Munich Heart Alliance, Munich, Germany
[i] Department of Statistics and Data Science, Faculty of Statistical Studies, Complutense University of Madrid, Madrid, Spain
[j] Genetics and Rare Diseases Research Division, Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, Italy
[k] Institute of Human Genetics, University Hospital Magdeburg, Magdeburg, Germany
[l] Endocrine, Bone Diseases, and Genetics Unit, Children's Hospital, Toulouse University Hospital, RESTORE INSERM UMR1301, Toulouse, France

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ABSTRACT

Introduction: Noonan syndrome (NS) is a rare genetic disorder caused by mutations in genes encoding components of the RAS/mitogen-activated protein kinase (MAPK) signalling pathway. Patients with NS exhibit certain characteristic features, including cardiac defects, short stature, distinctive facial appearance, skeletal abnormalities, cognitive deficits, and predisposition to certain cancers. Here, a clinical practice survey was developed to learn more about differences in the diagnosis and management of this disease across Europe. The aim was to identify gaps in the knowledge and management of this rare disorder.

Materials and methods: The European Medical Education Initiative on NS, which comprised a group of 10 experts, developed a 60-question clinical practice survey to gather information from European physicians on the diagnosis and clinical management of patients with diseases in the NS phenotypic spectrum. Physicians from three specialities (clinical genetics, paediatric endocrinology, paediatric cardiology) were invited to complete the survey by several national and European societies. Differences in answers provided by respondents between specialities and countries were analysed using contingency tables and the Chi-Squared test for independence. The Friedman's test was used for related samples.

Results: Data were analysed from 364 respondents from 20 European countries. Most respondents came from France (21%), Spain (18%), Germany (16%), Italy (15%), United Kingdom (8%) and the Czech Republic (6%). Respondents were distributed evenly across three specialities: clinical genetics (30%), paediatric endocrinology (40%) and paediatric cardiology (30%). Care practices were generally aligned across the countries participating in the survey. Delayed diagnosis did not emerge as a critical issue, but certain unmet needs were identified, including transition of young patients to adult medical services and awareness of family support groups.

Conclusion: Data collected from this survey provide a comprehensive summary of the diagnosis and clinical management practices for patients with NS across different European countries.

* Corresponding author. Institute of Medical and Molecular Genetics (INGEMM), Hospital Universitario La Paz Research Institute (IdiPAZ), Hospital Universitario La Paz, Paseo de la Castellana, 261, 28046, Madrid, Spain.
E-mail address: sixto.garcia@salud.madrid.org (S. García-Miñaúr).

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1. Introduction

Noonan syndrome (NS) is a relatively common developmental disorder with a roughly estimated incidence of 1/2000 to 1/2500 live births. The phenotype is highly variable and can involve multiple organ systems, including distinctive facial features, congenital heart disease, short stature, skeletal abnormalities, mild developmental delay/intellectual disability, and predisposition to myeloproliferative disorders (Roberts et al., 2013). NS belongs to a group of phenotypically overlapping genetic conditions caused by germline pathogenic variants in genes encoding components of the RAS/mitogen-activated protein kinase (MAPK) signalling pathway, collectively known as RASopathies (Tajan et al., 2018; Tartaglia et al., 2011). The diagnosis of NS is usually based on clinical features and diagnostic criteria have been proposed (van der Burgt et al., 1994). To date, a causative mutation in one of the twenty genes involved in NS and related disorders is identified in about 80% of patients with a suspected clinical diagnosis of NS (Grant et al., 2018). Genetic heterogeneity partly explains the phenotypic variability and genotype-phenotype correlations have been identified between specific pathogenic variants and certain clinical features, including pulmonic stenosis, hypertrophic cardiomyopathy, short stature, and haematological abnormalities defects (Cessans et al., 2016; Kouz et al., 2016; Motta et al., 2020; Pandit et al., 2007; Strullu et al., 2014; Tartaglia et al., 2002, 2003, 2007).

People with NS have different comorbidities and medical needs throughout their lifetime requiring management and follow up by different specialists, mainly clinical geneticists, (paediatric) cardiologists and endocrinologists. Approximately 70–80% of patients with NS have a congenital heart disease (50–60% pulmonary valve stenosis, 20–25% hypertrophic cardiomyopathy), and about 50–70% develop postnatal growth retardation (Roberts et al., 2013). Anticipatory medical care may allow prevention or early detection and treatment of medical complications associated with NS, hopefully improving the long-term outcome of these patients. To this end, medical guidelines have been developed to assist specialists involved in their care and to ensure that specific issues are addressed at different stages of development (NORD, 2019; BMJ Best Practice, 2018; DYSCERNE, 2011; Romano et al., 2010). However, adherence to these guidelines by specialists in different countries remains unknown. For the aforementioned reasons, patients with NS require multidisciplinary medical management that should be coordinated by a specialist familiar with this condition in order to avoid repetition of medical procedures, consultations, and hospital visits. Although the recommendations for medical management and follow-up are reasonably well known among paediatric specialists and are included in published guidelines, the situation is quite different for adult patients who nevertheless are affected by a variety of medical problems (Binder et al., 2012; Smpokou et al., 2012). There is also very limited information available on the natural history of NS. Development of national and international registries would provide valuable data to assess the efficacy and safety of specific medical interventions, such as growth hormone treatment or RAS/MAPK inhibitors, and to inform evidence-based recommendations for the management of patients with NS. The role of support groups should not be overlooked, as they provide valuable emotional support and medical information to families with a newly diagnosed child. They can be excellent advocates to raise awareness and to demand the specific needs required from the education and health services.

Little is known about differences in the diagnosis and clinical management of patients with NS across Europe. In order to obtain a 'snapshot' of current clinical practice, the European Medical Education Initiative on NS developed a clinical practice survey focusing on the diagnosis and clinical management of diseases within the NS phenotypic spectrum. The aim was to obtain feedback from relevant patient management centres across the continent, focusing on clinical geneticists, paediatric endocrinologists and paediatric cardiologists. The ultimate goal of this study is to assess disease management across Europe and to identify gaps in current clinical practice in order to improve patient care.

2. Material and methods

2.1. Survey development

The European Medical Education Initiative on NS was created in May 2020 and comprises a Steering Committee of ten experts from three clinical specialities closely involved in the treatment and management of patients with NS (clinical genetics, paediatric endocrinology, and paediatric cardiology). A 60-question clinical practice survey was developed during regular virtual meetings held between May and August 2020. A graphical representation of the survey architecture is shown in Fig. 1. To summarise here the structure and flow of the survey questions, the survey began by collecting demographic information on the respondent's location (city & country), clinical speciality, type of institution where they are primarily employed, and the number of patients they encounter on a yearly basis. Next, respondents were asked to answer general questions regarding the diagnosis of NS, with questions addressing age at referral, clinical characteristics, diagnostic assessments, and genetic testing. These questions were designed to be generally applicable to each of the three specialities targeted by the survey. Respondents then had the option to answer or skip specific questions relating to genetic confirmation of NS, including questions on patient referral, reimbursement of genetic testing costs, testing strategies, location of testing, time required for testing, prenatal diagnosis, and termination of pregnancy. While these were targeted towards clinical geneticists, they could potentially be viewed and answered by all respondents. General questions on the clinical management of patients were then asked to all specialities on several topics, including use of growth charts, treatment guidelines, and patient management in adulthood. Physicians could then answer or skip speciality-specific questions within cardiology or endocrinology sections, which again could be viewed and answered by each respondent. A general section at the end of the survey aimed to collect data on patient organizations/advocacy groups and quality of life. An open text box was included at the end to gather any general feedback from those who completed the survey. All questions included in the final version of the survey are provided in Supplementary Information.

2.2. Survey implementation and distribution

The survey was implemented for distribution in the Survey Monkey online platform, and physicians from the three specialities were invited to participate. Members of the Steering Committee contacted several societies for support to distribute a link to the survey within their regular newsletters. A list of the societies who assisted with survey distribution is provided in the acknowledgements. The survey was available for completion between August and November 2020. The first respondent completed the survey on 01 September 2020, and the last on 11 November 2020. Results provided in the current study describe responses to general questions relevant to all specialities and genetic diagnosis by clinical geneticists. Results from endocrinology and cardiology sections will be published separately (Edouard et al., 2021; Wolf et al., 2021).

2.3. Statistical analysis

For statistical analysis, results were directly exported in SPSS format from the Survey Monkey platform. For each question, the number of physicians who skipped the question as well as the number of those who estimated they could not answer the question were specified. Only the responses of physicians who gave an answer were considered in the statistical analysis (*i.e.*, those who selected 'Cannot answer' were excluded).

Differences between specialities and countries were assessed using

contingency tables and the Chi-Squared test for independence. The Friedman's test was used for related samples. Given the variable distribution of respondents across countries, responses were only compared between the most represented countries (countries with >10 respondents [France, Spain, Germany, Italy, the United Kingdom, Czech Republic]). For comparison of the responses from clinical geneticists in different countries, the four most represented countries with >10 respondents were France, Spain, Italy, and the United Kingdom.

All tests were two-tailed and P < 0.05 was considered significant throughout the study. Statistical analyses were performed using SAS software, version 9.4 (SAS Institute Inc., Cary, NC, USA) and SPSS Statistics for Windows, Version 25.0 (Armonk, NY: IBM Corp, Armonk, NY, USA).

3. Results

3.1. Respondent demographics

In total, there were 462 respondents from 25 countries. Of these, 98 were removed from the current analysis (81 respondents skipped the survey early after completing the demographics section, 6 were from outside the 28 countries in Europe that were selected for further analysis, and 11 were from other specialities [neonatology, neuropsychiatry, neurology, dysmorphology, general paediatrics, adult cardiology, foetal pathology]). This resulted in a core analysis set of 364 respondents from 20 European countries, of whom 40% were paediatric endocrinologists, 30% were paediatric cardiologists, and 30% were clinical geneticists. The majority (84%) of respondents came from six countries: France (21%), Spain (18%), Germany (16%), Italy (15%), United Kingdom (8%), and the Czech Republic (6%) (Fig. 2A and Supplementary Table 1). The distribution of specialists between the six most represented countries showed a significantly lower frequency of clinical geneticists in Germany and the Czech Republic (p < 0.0001), and paediatric cardiologists in Italy, United Kingdom and the Czech Republic (p < 0.0001); there was no significant differences for paediatric endocrinologists (p = 0.187).

Most physicians (73%) were based in university hospitals alone or in combination with other institutions, with 17% being based in general hospitals, and 11% in private clinics or independent practices (Fig. 2B). The proportion of physicians based in a university hospital did not differ according to the speciality (p = 0.139). In contrast, the distribution of specialists based in private clinics or independent practices was significantly higher for paediatric cardiologists and lower for clinical geneticists (p = 0.013).

Overall, most respondents (69%) see more than four patients with NS in an average year, with 16% seeing 11–20 patients, and 18% seeing >20 patients (Fig. 2C). When comparing results between specialities in the >20 patient category, the number of responses was significantly higher for clinical geneticists (30%) (p < 0.0001).

3.2. Patient diagnosis

Respondents were asked to answer how frequently patients are referred to them for NS from five age groups. When merging categories 'Frequently' (26–50%) and 'Most' (>50%), the majority of respondents were referred patients in the 'childhood (4–12 years)' age group (53%), followed by 'toddlerhood (1–3 years)' (38%) and 'infancy (<1 year)' (34%). The age at referral of patients suspected of NS differs between specialities. Although clinical geneticists received patients of the three main age groups, paediatric cardiologists mainly received infants, and paediatric endocrinologists children aged 4–12 years (p < 0.0001) (Fig. 3A).

The main clinical signs leading to a clinical diagnosis of NS were congenital heart defects (respondents selecting 'Frequently' or 'Most': 83%), characteristic facial features (72%), and short stature (66%). Other frequent clinical characteristics were also reported, notably chest deformities (27%), neonatal feeding difficulties/failure to thrive (23%), developmental delay (17%), lymphatic abnormalities (12%), and undescended testis in males (12%) (Fig. 3B).

Physicians were also asked about the baseline assessments that they would recommend for the different age groups once confident about the clinical diagnosis of NS (Supplementary Fig. 1). Although the answers to this question were difficult to analyse, it is important to note that for the 'All ages' group, 91% of respondents recommend performing electrocardiogram (ECG) and echocardiography, and between half to two-thirds of respondents recommend to perform Ear, Nose and Throat (ENT) and ophthalmology consultations, renal ultrasound, and to analyse complete blood count and coagulation profile.

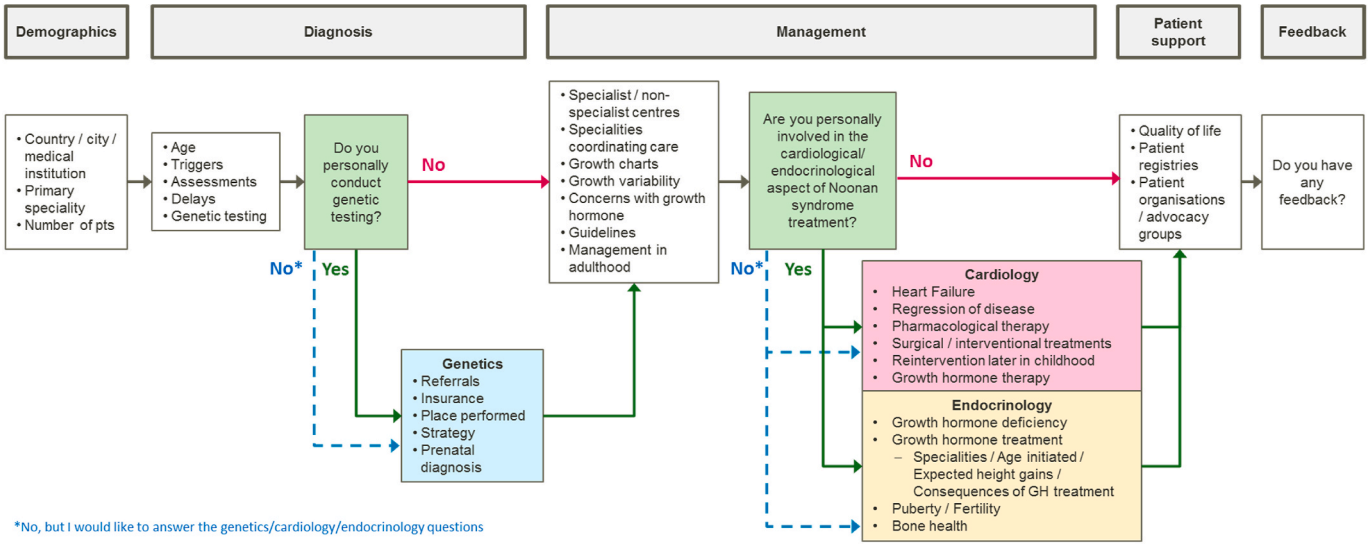

Fig. 1. Survey architecture.
Graphical representation of the survey structure and topics surveyed. White boxes show topics for questions that were relevant for individual specialities. Green boxes represent questions that allowed respondents to view, answer or skip sections that were or were not relevant to their speciality. Other coloured boxes represent speciality-specific questions for geneticists (blue), cardiologists (red) and endocrinologists (orange). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

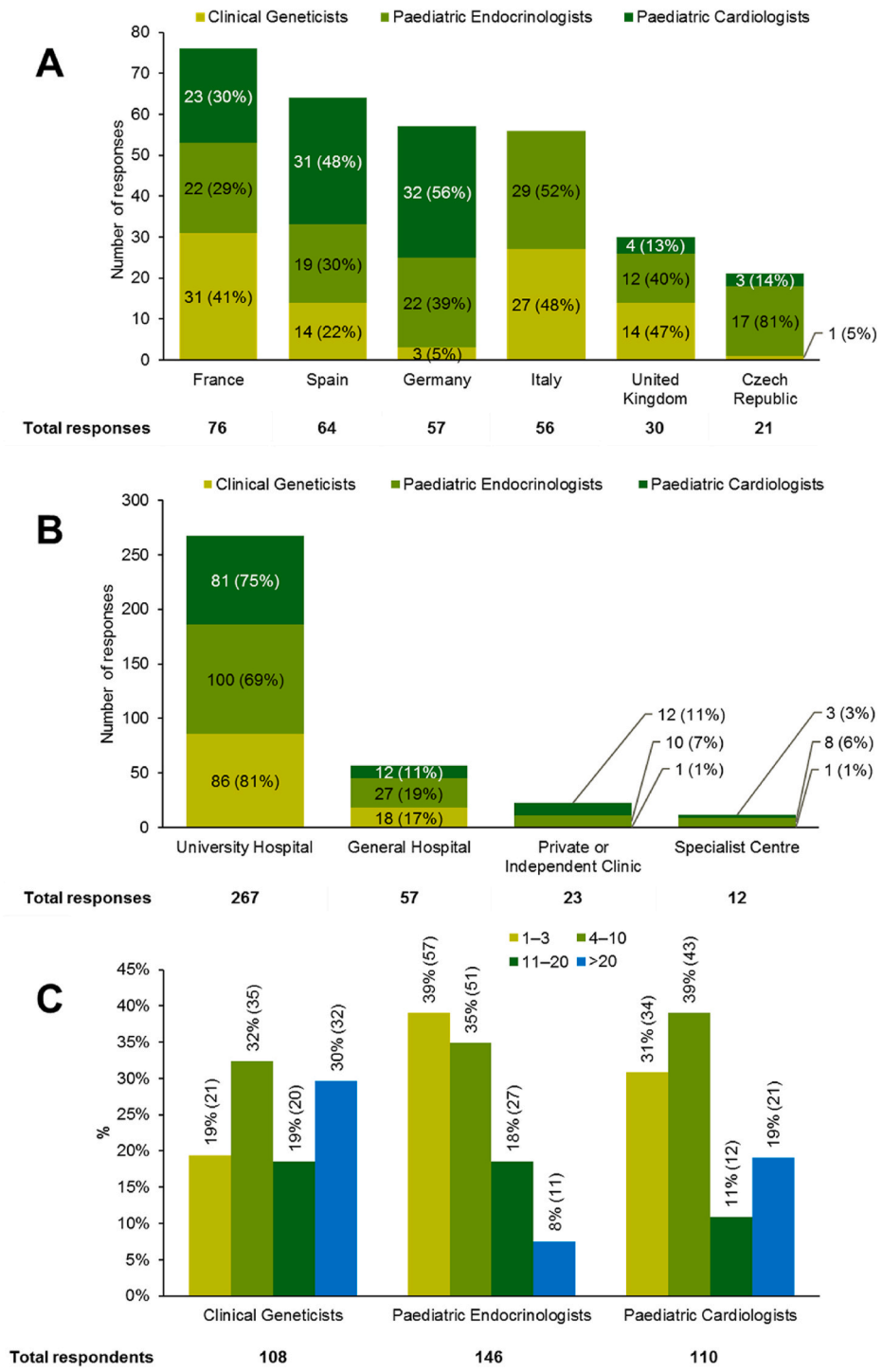

Fig. 2. Respondent demographics.
All respondents (n = 364) completed the following demographics questions:
A. What is your speciality/area of interest? Shown are the responses from the top six countries (n = 304) split by speciality (n [%]).
B. By which type of medical institution are you primarily employed? Shown are the responses from n = 359 respondents split by speciality (n = 5 who selected 'other' were excluded) (n [%]).
C. How many patients with Noonan syndrome or other clinically related disorders are seen by you/your department in an average year? Shown are the responses from all respondents (n = 364) as split by speciality (% [n]).

Most physicians from the three specialities (80%) agreed to conduct or order genetic testing even if the diagnosis of NS is clinically evident. However, it is important to note that 14% of physicians (8% of clinical geneticists, 21% of paediatric endocrinologists and 10% of paediatric cardiologists) were less likely to do so, and 5% selected 'cannot answer' for this question. There were significant differences between specialities with clinical geneticists being most likely to perform genetic testing, and paediatric endocrinologists being less likely (p = 0.004).

3.3. Genetic testing and prenatal diagnosis

For specific questions on genetic testing and prenatal diagnosis, only the responses from geneticists (n = 108) were analysed.

The vast majority of geneticists (96%) answered that genetic testing services are covered by their national health service or patient health insurance, without significant differences between countries. Consequently, for the majority of respondents (87%), problems with reimbursement of genetic testing were not a limiting factor for obtaining a correct genetic diagnosis.

Approximately half of the geneticists (48%) indicated that their genetic testing is performed in a genetics reference centre, with 35% saying testing is performed in a university setting, and only 10% in a private laboratory. The remaining 8% in the 'Other' category includes various options that are mostly university or public hospital-based

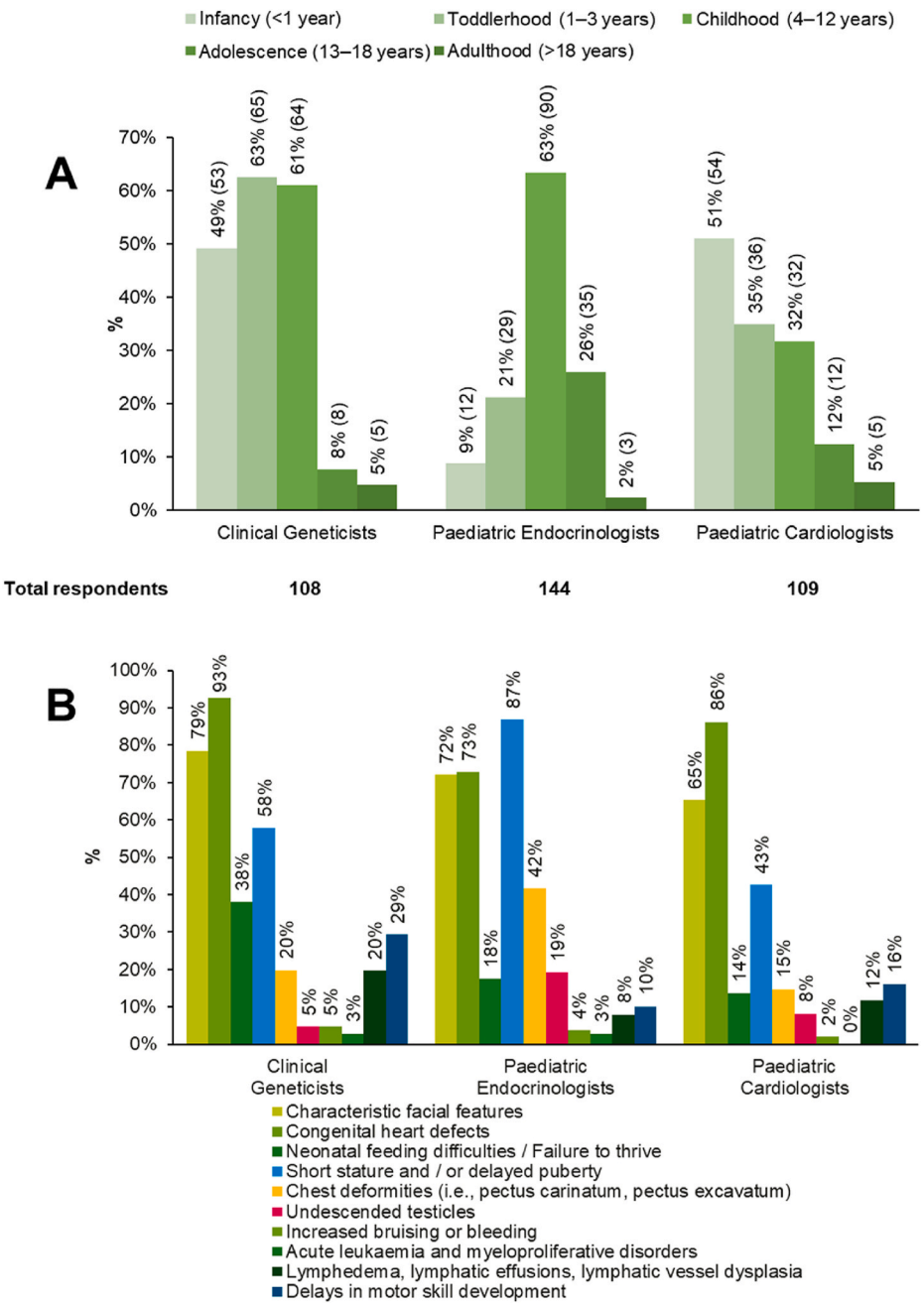

Fig. 3. Clinical diagnosis.
A. How frequently are patients referred to you from each age group for Noonan syndrome? This question was answered by n = 361 (99%) respondents. Physicians could select from the following choices for the five age groups: 'Never', 'Very Seldom', 'Occasionally', Frequently' and 'Most'. Shown are the results of merging 'Frequently' and 'Most' for each age group (% [n]).
B. In your experience, how frequently are the following characteristics a leading cause for a clinical diagnosis of Noonan syndrome? This question was answered by n = 362 (99%) respondents. Physicians could select from the following choices for each characteristic: 'Never', 'Very Seldom', 'Occasionally', Frequently' and 'Most'. Shown are the results of merging 'Frequently' and 'Most' for each age group (% [n]).

laboratories.

Geneticists were asked to rank from one to three the most common genetic testing strategy in their institution. A majority of respondents across all countries surveyed indicated that a multi-gene panel was the current approach of choice, with single gene selections coming in second, and exome sequencing currently a less standard option for confirming the diagnosis ($p < 0.0001$ using Friedman's test).

Regarding the estimated time between referral for a clinical genetic evaluation and final genetic confirmation of diagnosis, 40% of respondents said between 4 and 6 months, 30% between 7 and 12 months, 18% between 1 and 3 months, and 12% > 12 months. When categories were merged into two (≤6 months, 58%, and >6 months, 42%) there were no significant differences between countries ($p = 0.149$).

Two thirds (63%) of geneticists answered they would offer prenatal testing after appropriate counselling in cases where prenatal features of NS (*e.g.*, increased nuchal translucency, hydramnios, and cardiomyopathy) are observed together with a normal karyotype and absence of a family history. Otherwise, 23% would offer it under certain conditions, and 14% would not offer it at all. There were no significant differences between countries ($p = 0.110$).

One third of geneticists (28%) chose 'Cannot answer' when asked about the frequency of termination of pregnancy in their institution due to a prenatal diagnosis of NS following genetic testing. Of the remaining geneticists who answered this question, 12% answered 'Never (0%)', 42% 'Very seldom (1–5%)', 21% 'Occasionally (6–25%)', 14% 'Frequently (26–50%)', and 11% 'Most (>50%)'. The distribution of these categories across countries showed no statistically significant differences ($p = 0.341$). When categories were merged and regrouped into three ('Never or Very seldom' (≤5%), 'Occasionally' (6–25%), and 'Frequently or Most' (>26%)), 'Never or Very seldom' represented 54% with no significant differences between countries ($p = 0.118$).

The decision to terminate a pregnancy was strongly influenced by the

presence or absence of associated findings. Geneticists were asked how frequently families opt for termination of pregnancy when the following accompanying features are present: hydrops, cardiomyopathy, and congenital heart defects; a 'no other accompanying findings' option was also provided. For hydrops, the proportion of respondents answering with 'Frequently' or 'Most' ranged between 60% (United Kingdom) and 100% (Spain), with no significant differences between countries ($p = 0.065$). For cardiomyopathy, it was significantly higher in Spain (100%) ($p = 0.006$). For congenital heart defects, it was significantly higher in Spain (86%) and lower in United Kingdom (10%) ($p = 0.001$). There were no statistical differences between countries if there were no other associated findings ($p = 0.074$) (Fig. 4).

3.4. Management and follow-up

Of the respondents from the three specialities who could answer where patients are typically managed, 65% said 'specialist centres', and 31% said 'combination of specialist and non-specialist centres'. There were no statistically significant differences between specialities ($p = 0.134$) or countries ($p = 0.156$).

Physicians were also asked which clinical specialities are in charge of coordinating care in their institution, with the option to select more than one type of specialist. Results indicated that 65% of respondents selected paediatric endocrinologists, 54% clinical geneticists, 46% paediatric cardiologists, 30% primary healthcare physician, and 9% other. A small proportion (5%) selected 'Cannot answer'. There were significant differences between specialities: geneticists selected 'primary healthcare physicians and geneticists' more frequently than the other two specialities, and 'endocrinology' less frequently ($p < 0.0001$).

Regarding the guidelines used for the clinical management of patients with NS, the most commonly used were DYSCERNE (29%), British Medical Journal (BMJ) Best Practice (17%), Noonan Syndrome Support Group (NSSG) (15%) and National Organization for Rare Disorders (NORD) (10%). Nearly half of the respondents (45%) use other local, national, or international guidelines. However, it must be stressed that 9% of respondents do not follow any specific guidelines, and 10% selected 'Cannot answer'. There were significant differences between specialities with geneticists being most likely to use guidelines and paediatric cardiologists being less likely ($p < 0.0001$).

In most cases (64%), there is no dedicated consultation or transition clinic for patients transferring from child to adult services, without differences between specialities or countries. When asked about the specialists that are involved in the follow up of adult patients with NS (more than one option of specialist could be selected), 66% of respondents selected cardiologists, 41% endocrinologists, 36% clinical geneticists, 35% primary healthcare physician and 5% other. A significant proportion of cases (19%) selected "Cannot answer".

Heart defects are considered the most important factor affecting quality of life in infancy and toddlerhood. Growth retardation and learning disabilities become more important in childhood. Skeletal abnormalities, social issues and affective disorders add up in adolescence to the previous concerns in childhood. Social issues and affective disorders are the most important factors in adulthood (Fig. 5 & Supplementary Fig. 2).

Most respondents (74%) answered there were either no registries in their country or that they didn't know, without differences between specialities ($p = 0.339$). Approximately two thirds of respondents (59%) answered there was no national patient organization in their country. There were significant differences between specialities, with a higher rate of affirmative responses for the geneticists (69%) and lower rates for the cardiologists (23%) ($p < 0.0001$).

4. Discussion

This survey reports for the first time the current clinical practice for patients with NS across different European countries. Care practices were generally aligned across the countries participating in the survey. Delayed diagnosis did not emerge as a critical issue, but certain unmet needs were identified, including transition of young patients to adult medical services, coordination between specialists and awareness of family support groups.

4.1. Diagnosis

Most patients suspected of NS were referred during childhood, followed by toddlerhood and infancy. There were differences between specialities, with the geneticists seeing patients more frequently during infancy and toddlerhood, the paediatric cardiologists during infancy, and the paediatric endocrinologists during childhood. These results were expected given the role of geneticists in the diagnosis of these patients irrespective of age and severity of the disease; paediatric cardiologists and paediatric endocrinologists usually follow patients with heart defects (often diagnosed at birth or during infancy) or short stature (becoming obvious in childhood), respectively.

The main clinical signs at referral are distinctive/suggestive facial features, cardiac defects and short stature, which are the cardinal

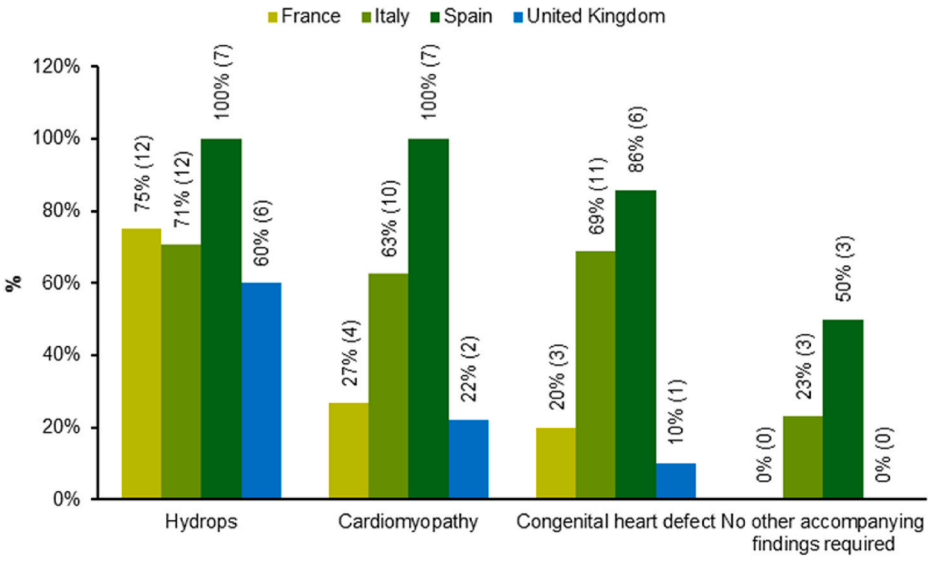

Fig. 4. How frequently do families opt for termination of pregnancy following genetic confirmation of Noonan syndrome, if the following accompanying features are present in the foetus? Shown are the answers from n = 51 (59%) clinical geneticists split by the top four countries. Physicians could select from the following choices for each accompanying feature: 'Never', 'Very Seldom', 'Occasionally', Frequently' and 'Most'. Shown are the merged results for 'Frequently' and 'Most' (% [n]).

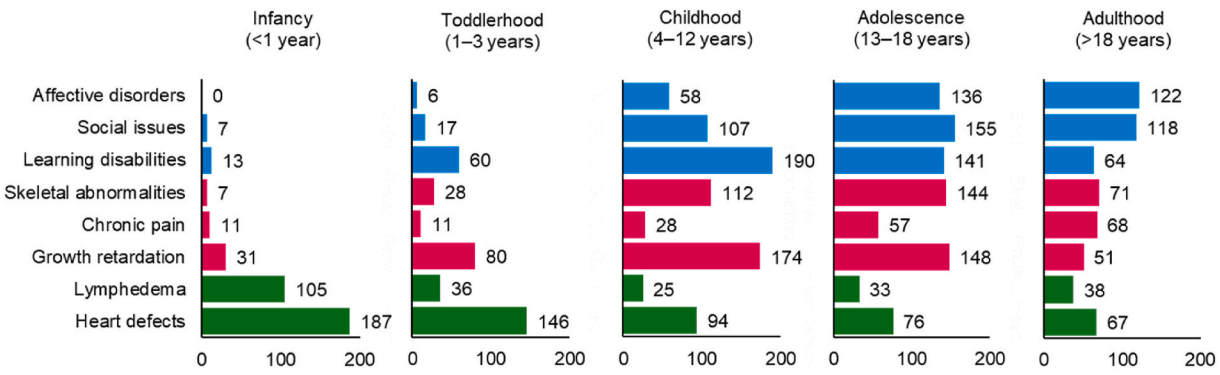

Fig. 5. What are the most important factors in each age group that affect quality of life in patients with Noonan syndrome? Shown are the answers from n = 242 (66%) physicians for affective disorders/social issues/learning disabilities (blue), skeletal/pain/growth (pink) and lymphedema/heart (green). Note that respondents could provide answers in one or more categories. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

features proposed in the diagnostic criteria defined by van den Burgt and colleagues (van der Burgt et al., 1994). This scoring system, which was established before the genes involved in NS were identified, includes typical or suggestive dysmorphic features, pulmonary valve stenosis and/or typical ECG (*i.e.*, left axis deviation and dominant S-waves in the left precordial leads), short stature, skeletal abnormalities (*i.e.*, pectus carinatus/excavatum, broad thorax), developmental delay/learning disability, cryptorchidism in males, lymphatic dysplasia, and family history of NS. Interestingly, although not included in this scoring system, this survey highlights important signs at referral, notably neonatal feeding difficulties and failure to thrive. Feeding difficulties (such as a weak sucking, prolonged feeding time or recurrent vomiting) are frequently reported in infants with NS (about three quarters of infants) and may require tube feeding in about one quarter of cases (Draaisma et al., 2020; Sharland et al., 1992; Shaw et al., 2007). These feeding difficulties, which usually resolve in the first months/years of life, are often associated with failure to thrive. Failure to thrive was present in the scoring system proposed in 1981 by Duncan and colleagues (Duncan et al., 1981). However, this scoring system, which included 21 items, was abandoned because too cumbersome to apply in practical use. These results underlie the need to update scoring systems on the clinical and molecular standpoints.

Most physicians agreed to conduct genetic testing, even if the diagnosis of NS is clinically evident, which is advisable given the medical implications of the genotype-phenotype correlations and the difficulties to predict the genotype from the facial phenotype alone (Allanson et al., 2010; Grant et al., 2018; Tartaglia et al., 2011). However, it is important to note that 14% of physicians (of whom 21% of paediatric endocrinologists) were less likely to conduct genetic testing. Insurance and reimbursement of expenses is not a limiting factor because the vast majority of geneticists (96%) answered that genetic testing services are covered by their national health service or patient health insurance. However, there is an unexpectedly long turnaround time for genetic testing with almost half of the respondents reporting a time of >6 months. There is emerging evidence of genotype-phenotype correlations between specific variants in RASopathy genes and certain clinical features, including cardiac, growth, and haematological defects (Cessans et al., 2016; Kouz et al., 2016; Motta et al., 2020; Pandit et al., 2007; Strullu et al., 2014; Tartaglia et al., 2002, 2003, 2007). Further prospective studies are needed to investigate possible genotype-phenotype correlations in terms of predisposition to tumour development or efficiency and safety of growth hormone treatment. In order to answer this key question, all physicians must be aware of the importance of a genetic diagnosis in all NS patients. There is also a need for the creation of coordinated European registries to collect data on the evolution of these patients, including the medical complications that may arise during their lives.

4.2. Prenatal diagnosis

The survey gives us precious information on the application and consequences of prenatal diagnosis of NS across different European countries. No significant differences were observed in the supply of and access to prenatal genetic diagnosis. However, this survey did only ask under which conditions genetic specialists would offer prenatal genetic testing, and it cannot provide an estimate about the actual frequencies or numbers of prenatal testing that are actually performed in the participating European countries. There are significant differences in the reported termination rates of affected pregnancies with associated congenital heart disease, which was higher in Spain and lower in the United Kingdom. A selection bias is possible as couples who would not consider terminating an affected pregnancy would decline prenatal genetic testing. However, these differences may also be due to differences in the organization and provision of prenatal diagnosis and genetic counselling between countries.

Prenatal manifestations of NS may include polyhydramnios, abnormalities of the lymphatic system (*i.e.*, increased nuchal translucency, cystic hygroma, distended jugular lymphatic sacs, pleural and/or pericardial effusion, ascites, *hydrops fetalis*), cardiac and/or renal anomalies. The frequency of these prenatal manifestations in cases of postnatally confirmed NS is estimated at 21–50% (Bakker et al., 2011; Baldassarre et al., 2011; Menashe et al., 2002; Myers et al., 2014). An evolving phenotype has been suggested during *in utero* and postnatal life whereby characteristic cardiac abnormalities (*i.e.*, pulmonary stenosis, hypertrophic cardiomyopathy) may be difficult to identify in the prenatal period (Achiron et al., 2000). Although a possible relationship between presence of prenatal manifestations and postnatal outcome has been suggested, there are discrepancies between studies (Baldassarre et al., 2011; Gaudineau et al., 2013). Depending on the prenatal manifestations and the extent of the genetic study performed, a molecular diagnosis of NS or other RASopathy is done in 9–17% of foetuses with increased nuchal translucency associated with a normal karyotype (Croonen et al., 2013; Lee et al., 2009; Scott et al., 2021). Moreover, alterations in RASopathy genes have been detected in 29% of non-immune *hydrops fetalis* cases studied by whole exome sequencing (Sparks et al., 2020), and RASopathy gene pathogenic variants are found in 16% of foetuses with cystic hygroma, with a higher prevalence in case this specific feature is persistent during the second trimester (21%) or when it is associated with other findings (*e.g.*, cardiac disease) (28%) (Scott et al., 2021). This led to proposing that, after a normal chromosomal microarray analysis, genetic testing for NS or a related disorder should be performed for any foetus when findings suggestive of lymphatic dysplasia or cardiac disease are found alone or in association, except for isolated increased nuchal translucency below 6 mm or isolated increased nuchal fold (Scott et al., 2021), or with nuchal

translucency ≥3.5 mm in association with other ultrasound anomalies (*e.g.*, distended jugular lymph sacs, *hydrops fetalis*, polyhydramnios, pleural effusion, ascites, cardiac defects and renal anomalies) (Stuurman et al., 2019). Because of the variable phenotypic expressivity and genetic heterogeneity, it is often difficult to predict the outcome. Appropriate genetic counselling, preferably by a clinical geneticist, is required prior to prenatal genetic testing.

4.3. Management and follow-up

This survey helps us to identify important gaps in the management of patients with NS, notably the lack of recognized international guidelines, specific registries, transition clinics and national patient organizations.

No consensus was observed on the use of guidelines for the management of patients with NS. Only one-third of physicians (mainly clinical geneticists) use published NS-specific guidelines (NORD, 2019; BMJ Best Practice, 2018; DYSCERNE, 2011; Romano et al., 2010), whereas nearly half of the respondents use other local or national guidelines, and almost 20% of physicians do not follow any specific guidelines or selected 'Cannot answer'. The current published guidelines have important limitations. Indeed, among the four published guidelines (NORD, 2019; BMJ Best Practice, 2018; DYSCERNE, 2011; Romano et al., 2010), two were published in 2010 and not updated since (DYSCERNE, 2011; Romano et al., 2010). All of them were established by a small number of specialists mainly originating from the United Kingdom or United States, and these guidelines do not meet the formal criteria of a guideline (*i.e.* no indication of evidence level, no standardized consensus process). The development and the validation of international guidelines by different specialists from different countries is essential to improve the management of patients with NS and other RASopathies worldwide.

Two thirds of respondents answered that there is no dedicated consultation or transition clinic for patients transferring from child to adult services, without differences between specialities and countries. Moreover, regarding the specialists involved in the follow up of adult patients with NS, the responses were highly variable between specialities. In contrast to what has been done for other syndromes such as Turner syndrome (Gawlik and Malecka-Tendera, 2014), no study has been published on transition to adult health care in NS. It should be stressed that most medical problems that occur in NS during childhood should be followed up into adulthood, including tumour predisposition, cognitive and behavioural difficulties, cardiac and musculoskeletal defects. Furthermore, several recent studies suggest that adult patients with NS may be at risk of infertility (Moniez et al., 2018), osteopenia (Baldassarre et al., 2017), and metabolic disturbance (Paccoud et al., 2021). Although the medical complications of NS are well known during childhood and adolescence, to date only a few studies have reported the medical complications and educational outcomes in adults with NS (Binder et al., 2012; Smpokou et al., 2012). In this survey, according to physicians, the most important factors that affect quality of life in adulthood are social and affective issues. The medical management and follow-up of adult patients with NS requires the participation of a multidisciplinary team comprising geneticists, cardiologists, endocrinologists, fertility specialists, behavioural health experts and social workers. A coordinated transition process from paediatric to adult care with a designated coordinator is needed in order to avoid gaps in medical care, to ensure appropriate follow-up, and to improve our knowledge on the long-term consequences of the disease. National support groups may also help in follow-up during adulthood. In this context, it is also unsettling that over half (59%) of the physicians were not aware of a local or national NS support group.

4.4. Study limitations

This survey is original and includes a large number of responses from physicians closely involved in the management of patients with NS; however, it has limitations and potential selection biases. Although the number of paediatric endocrinologists was slightly higher, respondents were overall evenly distributed across clinical genetics (30%), paediatric endocrinology (40%) and paediatric cardiology (30%), making the comparison between these specialities possible. In contrast, specialists were not homogeneously distributed across countries. As a result, specialist responses were only compared among the most represented countries, which may not be representative of other European countries. Moreover, three quarters of specialists were based in university hospitals alone or in combination with other institutions. This predominance of university hospitals is probably explained by the fact that most children with NS are followed up in tertiary centres alone or in combination with other institutions (96%); however, a possible recruitment bias of survey responders is possible, the physicians working in university hospitals being more likely to respond the survey than those working in independent practice. Finally, this survey does only reflect the opinions and experience of physicians who responded to this survey and it remains unclear what proportion of patients is actually covered by the responses we received.

5. Conclusions

The results of the survey show an apparent high degree of concordance in medical care practices across participating countries. The main goal of this survey is to identify weak/missing aspects in medical management of NS patients in childhood and adulthood. According to the results, the main findings could be summarized as follows:

- Genetic testing is provided equitably by national health services and according to clinical and molecular genetic standards (testing strategy, time to results).
- There is a need to ensure adequate genetic counselling prior to prenatal genetic testing (significant differences in termination rates between countries).
- Coordination between specialists involved in the management and follow up of paediatric NS patients needs to be improved.
- There is a lack of specialized clinics to ensure smooth transition to adult medicine avoiding gaps in medical care (transition clinics).
- There is no consensus in the use of medical guidelines and a lack of validated international guidelines.
- Specific registries that could provide valuable information on the medical needs and long-term outcome of NS patients are lacking.
- There is a lack of awareness and limited collaboration with NS support groups.

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Author contributions

SGM., E.BW, AV, GS, JL, IÖS, CMW, MT, MZ and TE contributed to development of the survey questions, analysis of the results, and drafting of the manuscript. EOC performed statistical analysis. All authors have read and approved the final version of the manuscript for submission.

Data availability statement

All of the data supporting the results presented in this paper are available on request.

Declaration of comepting interest

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Appendix A. Supplementary data

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