NICOLA Conference Presentations

2014

Date	14th March 2014
Event/location	Vision TRG Conference, Malone Lodge Hotel, Belfast
Title	Development of Ultra-wide field colour fundus photography grading scheme for use in epidemiological studies to evaluate peripheral lesions
Authors	Quinn, Nicola B.[1]; Aslam, Asma[2]; Lengyel, Imre[2]; Peto, Tunde[3]; Hogg, Ruth
Purpose	Develop a grading scheme to classify peripheral retinal lesions suitable for use in a large epidemiological study
Methods	A grading scheme was developed based on a combination of the Moorfield's grid and the Wisconsin age-related maculopathy grading scheme (WARMGS) grid; additionally eight subfields beyond the standard macular area were defined. A systematic review of potential retinal lesions identified seven key lesions. Validation and inter-grader repeatability was undertaken by two independent graders using images from the Reykjavik Eye Study. Optomap colour images of fifty participants (1 image per eye) were selected randomly from the Reykjavik Eye Study. Percentage of lesions located in the various zones was calculated to inform on optimal design of a peripheral retinal grid.
Results	Agreement for identification of peripheral lesions ranged from 60%-100% (Kappa 0.48-1.0). Agreement was highest for presence or absence of individual retinal lesions (97%-100%, Kappa 0.92-1.0). Greatest variability was for presence of drusen in the various zones (60%-99%), Kappa 0.47-0.70).
Conclusion	In developing a grid for grading peripheral lesions, our findings suggest that the area beyond the traditional WARMGS macula grid should include zones to separate the mid periphery from the far periphery and also include subfields that respect the midline. Therefore we have proposed a grid that includes 16 peripheral sub-fields. This grading scheme showed low variability between graders and therefore should prove suitable for use in a large epidemiological study.
Abstract published	No

Date	7th May 2014
Event/location	ARVO Conference, Orlanda, Florida
Title	Refining the ultra-wide field colour fundus photography grading scheme for use in epidemiological studies

Authors	Quinn, Nicola B.[1]; Aslam, Asma[2]; Lengyel, Imre[2]; Peto, Tunde[3]; Hogg, Ruth E.[1]
Purpose	To develop a grading scheme to classify peripheral retinal lesions suitable for use in a large epidemiological study.
Methods	A grading scheme was developed based on a combination of the Moorfield's grid (which is derived from the International Classification for age-related macular degeneration (AMD)) and the Wisconsin age-related maculopathy grading scheme (WARMGS) grid. These macula centred grids were altered in order to capture information from the peripheral retina. Eight subfields beyond the standard macular area were defined using an extension of the WARMGS sections together with vertical and horizontal lines in both the mid and far periphery. A systematic review of potential retinal lesions identified the following lesions for presence/absence evaluations: choroidal neovascular membrane, geographic atrophy, floaters, naevi, retinal tears, white without pressure and haemorrhages. Drusen and RPE changes were evaluated in terms of which retinal zone they were noted in. Validation and inter-grader repeatability was undertaken by two independent graders using images from the Reykjavik Eye Study. Optomap colour images of fifty participants were selected randomly from the Reykjavik Eye Study, providing 100 images to grade. Percentage of lesions located in the various zones was calculated to inform on optimal design of a peripheral retinal grid.
Results	Agreement for identification of peripheral lesions ranged from 60%-100% (Kappa 0.48-1.0). Agreement was highest for presence or absence of individual retinal lesions (97-100%, Kappa 0.92-1.0). Greatest variability was for presence of drusen in the various zones (60%-99%, Kappa 0.47-0.70). Drusen were most frequently located superiorly (68%). RPE changes were also seen most commonly in the superior sections (63%), in the most peripheral zones (81%) and in those subfields closest to the midline (75%).
Conclusion	In developing a grid for grading peripheral lesions, our findings suggest that the area beyond the traditional WARMGS macula grid should include zones to separate the mid periphery from the far periphery and also include subfields that respect the midline. Therefore we have proposed a grid that includes 16 peripheral sub-fields. This grading scheme showed low variability between graders and therefore should prove suitable for use in a large epidemiological study.
Abstract published	Yes
Abstract reference	Investigative Ophthalmology & Visual Science April 2014, Vol.55, 4818.

Date	7th May 2014
Event/location	ARVO Conference, Orlando, Florida

Title	Can Heidelberg MultiColor images be used interchangeably with color fundus photography for grading age-related macular degeneration features?
Authors	Graham, Katie[3]; Larkin, Patrick[2, 3]; Muldrew, Katherine Alyson[2, 3]; Silvestri, Vittorio [1, 3]; Young, Graham[1, 2]; McIntyre, Philip[1]; McAtamney, Helen[1]; Hogg, Ruth
Purpose	To characterize the appearance of age-related macular degeneration features (AMD) on Heidelberg MultiColor images(MCI) and then systematically compare their appearance with color fundus photography (CFP).
Methods	Study design: Observational case series. Participants: 30 patients attending Macular clinics in Belfast and 30 participants from the Northern Ireland Cohort for the Longitudinal study of aging (NICOLA). Images were obtained after dilation using both CFP and cSLO MCI (Heidelberg Engineering, Germany) using standardized protocols. Color fundus photographs were assessed and clinical features of AMD noted (hard drusen, soft drusen, reticular pseudodrusen, geographic atrophy, haemorrhage and fibrosis), the constituent images from the cSLO imaging were assessed in turn (infrared(IR), green reflectance(GR), blue reflectance(BR) and composite MultiColor) and the presence or absence of the features noted on CFP was determined. Features present on MultiColor and absent on CFP were also noted. Test characteristics were determined and a matrix describing the appearance of AMD features on the different images was constructed. Examples of artefact's were also collected.
Results	A total of 99 eyes with gradable images were available for comparison (56 eyes from patients and 43 eyes from NICOLA study participants). Using CFP as the gold standard, sensitivity values for MCI ranged from 100% for fibrosis to 68% for soft drusen. Specificity values were high (95%+) for all features except hard drusen (75%). For all AMD features except haemorrhage there were instances where features were noted on MCI but not on CFP. When features were present on MCI their edges usually appeared more distinct than on CFP.
Conclusion	Although sensitivity and specificity values were high for most AMD features it is unlikely that these technologies could be used interchangeably. Careful interpretation is also required given the different appearance of features on CFP and MCI. Given the improved definition of features on MCI it may prove most useful in situations where measurement of lesion size is important.
Abstract published	No

Date	October 2014, November 2014
Event/location	October: Paul B. Beeson Career Development Awards in Aging

	Research Program Annual Meeting, San Diego, US, November: Centre of Excellence Away Day, NI
Title	Diet, retinal microvascular health and cognitive decline and dementia risk: the NICOLA and TILDA studies
Authors	Charlotte E Neville[1], Michelle C McKinley[1], Gareth J McKay[1], Frank Kee[1], Ian S Young[1], P Passmore[1], Chris R Cardwell[1], Rose Anne Kenny[2], Jayne V Woodside[1] [1]Centre of Excellence for Public Health, Queen's University Belfast, Northern Ireland, [2]The Irish Longitudinal Study on Ageing, Trinity College Dublin, Ireland.
Purpose	In the developed world, as life expectancy increases and birth rate declines, the proportion of older people is increasing. As the proportion of older people increases, so will chronic disease incidence. The increasing prevalence of cognitive decline and dementia, and the impact on healthcare systems is a major concern. In 2010, it was estimated that there were 35.6 million people with dementia worldwide with figures estimated to approximately double every 20 y. The ability to identify those at high risk of dementia at an early stage using non-invasive methodology will be important (e.g. by retinal microvascular assessment), whilst strategies to reduce age-related morbidity and chronic disease prevalence will encourage healthy ageing, and have financial and societal benefits. Dietary factors, including fruit and vegetable (FV) intake, have been suggested to play a role in promoting healthy ageing and reducing the risk of cognitive decline. Accurate assessment of dietary intake in older populations is therefore vital to determine this potential role of diet in promoting healthy ageing. However, accurate estimation of dietary intake is difficult, with methods commonly employed (e.g. food frequency questionnaires (FFQ), 24-h recalls and food diaries) each being associated with error. Using multiple dietary assessment methods and/or biomarker approaches may provide a more accurate estimate of true dietary intake, but this has not yet been tested in older populations. The current study explores the association between FV intake, retinal microvascular health and cognitive decline and dementia risk in the Northern Ireland Cohort Longitudinal Study of Ageing (NICOLA) (8,500 subjects, >50 y) and The Irish Longitudinal Study on Ageing (TILDA) (8,504 subjects, >50 y) studies. The study has four aims: 1) to validate the dietary assessment methodology used in NICOLA, using nutritional biomarkers, 2) to test other potential dietary assessment methods that may be particularly suited for older populations, 3) to determine the association between FV status and cognitive decline and dementia risk (cross-sectionally in NICOLA, longitudinally in TILDA) and, 4) to explore the use of retinal microvascular health assessment as a marker of cognitive decline and dementia risk in TILDA.

Methods	The study aims will be addressed by firstly conducting a validation of the FFQ, currently being used in NICOLA, against a 4-day food diary and a panel of biomarkers of FV intake, as objective biological indicators. Secondly, we will conduct a comparison of the multiple 24-h recall method, recently reported to be of use in an older population, with a 4-day food diary in a sub-sample of the NICOLA cohort. Thirdly, we will measure a panel of FV intake biomarkers in both NICOLA and TILDA which will allow us to analyse the association between FV intake biomarkers and measures of cognitive function in NICOLA (cross-sectional), and cognitive decline and dementia risk in TILDA (longitudinal) and to analyse the association between FV intake biomarkers and retinal microvascular parameters, as a possible indicator of cognitive decline. Finally, we will examine the association between retinal microvascular parameters and cognitive decline and dementia risk in TILDA.
Conclusions	This research is aimed at health and social policy makers involved in the promotion of healthy ageing. This research will correct the lack of dietary validation studies in older adults to date. It will also unravel the potential role of diet in healthy ageing and will ultimately lead to appropriate, evidence-based, dietary guidelines for older people to promote healthy ageing, in the context of an ageing population worldwide.

2015

Date	April 2015
Event/location	CARDI (Centre for Aging Research and Development in Ireland; currently known as IPH-Ageing Division) International Scientific Meeting and Leadership Event, Dublin
Title	Diet and cognitive decline and dementia risk
Authors	Neville, C.

2016

Date	19th February 2016 and 3rd May 2016
Event/location	Vision TRG Conference, The Wellcome-Wolfson Building, QUB, Belfast (February) and ARVO Conference, Seattle Washington (May)

Title	Do peripheral retinal lesions impact the vitreo interface in the posterior pole?
Authors	Quinn, Nicola B.1**;** Graham, Katie1; Elliot, David1; Hennessy, Riona1; Wright, D.M.2; Muldrew, Alyson1; Chakravarthy, Usha1; Peto, Tunde3; Hogg, Ruth E.1, NICOLA Study Group
Purpose	To determine the association between peripheral retinal lesions and the presence of vitreomacular adhesions
Methods	Ultra-wide field retinal images (Optomap 200 TX) and corresponding Heidelberg Spectral-Domain OCT retinal images were obtained Northern Ireland Cohort of the Longitudinal Study of Aging (NICOLA Study) participants. Images from 511 participants were assessed. The vitreomacular interface (VMI) was graded for the presence or absence of a vitreomacular adhesion (VMA) using a standardised protocol. The Optomap images were graded for the presence of 16 common retinal lesions, (hard and soft drusen, retinal pigment epithelial (RPE) changes, chorioretinal atrophy, bone spicules, haemorrhages, bear tracks, pavingstone degeneration, naevi, white without pressure, retinoschisis, congenital hypertrophy of the RPE (CHRPE), geographic atrophy (GA), choroidal neovascularisation (CNV), retinal hole and ungradeable area) using the Manchester Grid, which covers the image with 400 boxes, each approximately one disc area in size. Chi-squared tests were used to assess the association between presence of lesions in either the peripheral or central retina and status of the vitreous interface.
Results	960 Optomap and 942 OCT gradeable images were available for analysis. Participants ranged in age from 42 to 96 years (mean 64 years. SD 9.2), 47% were male. Prevalence of VMA within participants in this study was 70% with VMA being relatively evenly distributed between men (70%) and women (68%). Participants with RPE irregularities in their peripheral retina were less likely to have VMA present than those without. In the posterior pole those with hard drusen (p=0.05) or any stage of AMD (p=0.06) tended to be more likely to have VMA present than those without.
Conclusion	RPE irregularities in the periphery appears to be protective for VMA in the posterior pole whereas AMD features occurring in the macular area increase the risk of VMA.
Abstract published	No

Date	3rd May 2016
Event/location	ARVO Conference, Seattle, Washington
Title	Multimodal imaging for geographic atrophy; is colour fundus photography still our gold standard?
Authors	Graham, Katie[3]; Larkin, Patrick[2, 3]; Muldrew, Katherine Alyson[2, 3]; Silvestri,

	Vittorio [1, 3]; Young, Graham[1, 2]; McIntyre, Philip[1]; McAtamney, Helen[1]; Hogg, Ruth E
Purpose	To describe and characterize the appearance of age-related macular degeneration (AMD) geographic atrophy(GA) lesions on Heidelberg MultiColor images(MCI) and then systematically compare their appearance with color fundus photography (CFP).
Methods	Study design: Observational case series. Participants: 30 patients attending Macular clinics in Belfast and 30 participants from the Northern Ireland Cohort for the Longitudinal study of aging (NICOLA). Images were obtained after dilation using both CFP and cSLO MCI (Heidelberg Engineering, Germany) using standardized protocols. Color fundus photographs were assessed first, and the following sequence of lesion identification was followed: presence or absence of an AMD lesion (active or inactive) and presence or absence of GA. GA lesions were further categorized into atrophy type as follows; inside-lesion, peri-lesion, outside-lesion, combination of inside- and peri-lesion, combination of inside- and outside-lesion, combination of peri- and outside-lesion, and combination of inside-, peri-, and outside-lesion atrophy. Each GA lesion subtype was assigned a numerical code and graded as present or absent. The constituent images from the cSLO imaging were assessed in turn (infrared(IR), green reflectance(GR), blue reflectance(BR) and composite MultiColor) and the presence or absence of lesion, GA and GA subtypes as outlined previously. Features present on MultiColor and absent on CFP were also noted. SD-OCT images were assessed where necessary and when available (OCT capture not a necessity under original imaging protocol). Test characteristics were determined and a matrix describing the appearance of AMD features on the different images was constructed. For image sets showing discordance (GA present on MCI and not on CFP, the presence of GA was confirmed using corresponding OCT where available).
Results	A total of 99 eyes with gradable images were available for comparison (56 eyes from patients and 43 eyes from NICOLA study participants). Cross-tabulation of GA type for each imaging modality was computed. Using CFP as the gold standard, sensitivity for MCI for all GA types was high (96%). Highest sensitivity values were found for inside-lesion atrophy (100%), compared to relatively low values for inside-lesion (40%) and outside-lesion atrophy (50%). For the presence or absence of an AMD lesion, sensitivity was high for MCI compared to CFP (80.56). Specificity values were high (91%+) for all GA subtypes and presence or absence of AMD lesion. It was noted that in all GA detected on MCI, borders and identification had superior definition in comparison to CFP.
Conclusion	Sensitivity and specificity values were high for MCI compared to CFP for the detection of AMD lesion, GA and GA subtypes, with the exception of inside- and outside-lesion atrophy. The improved definition of GA borders on MCI means it has high potential to become a key imaging modality in both clinical and research assessment of patients with GA. However, the interpretation of MCI must be considered carefully due to altered colour appearance in comparison to conventional CFP.
Abstract published	No

2017

Date	8th May 2017
Event/location	ARVO Conference, Baltimore, Maryland
Title	Prevalence and characteristics of peripheral retinal lesions in an ageing population.
Authors	Quinn, Nicola B; Wright, D.M; Peto, Tunde; Cruise, S.M; Young, I.S.; Kee, Frank, Chakravarthy, Usha; Hogg, Ruth E.
Purpose	To determine the prevalence and spatial distribution of peripheral retina lesions and their associated risk factors in a population based sample of ageing individuals.
Methods	Ultra-wide field retinal images (Optomap 200 TX) were obtained from the Northern Ireland Cohort of the Longitudinal Study of Aging (NICOLA Study) participants. Images from the first 1468 participants were assessed. The Optomap images were graded for the presence of 16 common retinal lesions, (hard and soft drusen, retinal pigment epithelial (RPE) changes, chorioretinal atrophy, bone spicules, haemorrhages, bear tracks, pavingstone degeneration, naevi, white without pressure, retinoschisis, congenital hypertrophy of the RPE (CHRPE), geographic atrophy (GA), choroidal neovascularisation (CNV), retinal hole and ungradeable area) using the Manchester Grid, which covers the image with 400 boxes, each approximately one disc area in size. Descriptive statistics were used to describe the prevalence and spatial distribution of the retinal lesions. Generalised estimating equations were used to determine risk factors associated with each retinal lesion.
Results	A total of 3044 images were available for analysis. Participants ranged in age from 40 to 96 years (mean 64 years. SD 9.02), with male and females making up 48.4% and 51.6% of the sample respectively. Prevalence rates ranged from 0.1% for CNV and snailtrack degeneration to 99.8% for hard drusen. The prevalence of lesions were WWOP (11.6%), RPE changes (17.8%), haemorrhages (6.9%), chorioretinal atrophy (8.2%), naevi (10.9%) and soft drusen (8.2%). Confounder adjusted analysis revealed that soft drusen, RPE changes, naevi and chorioretinal atrophy were associated with increasing age. Haemorrhages were associated with a history of cardiovascular disease. Hard drusen was predominantly seen superiorly, RPE changes in the far nasal periphery and WWOP in the far temporal periphery.
Conclusion	Peripheral retinal abnormalities are common in the older population with varying prevalence rates. Some peripheral lesions appear to show distinct spatial patterns whereas other occur throughout the retina. The mechanisms underlying the spatial distribution are not well understood and

	deserve further investigation.
Abstract published	Yes
Abstract Ref	Investigative Opthalmology and Visual Science 58(8), 1

Date	June 2017
Event/location	Nutrition Society (Irish Section) Annual Meeting, QUB, Belfast
Title	Validity of fruit and vegetable intake assessed by a food frequency questionnaire (FFQ) in older adults: the Northern Ireland Cohort for the Longitudinal Study of Aging (NICOLA).
Authors	CE Neville, MC McKinley, F Kee, IS Young, CR Cardwell and JV Woodside
Purpose	Accurately assessing dietary intake in older populations is vital to determine the potential role of diet in healthy ageing. However, accurate estimation of dietary intake is difficult, with commonly employed methods each being associated with error [(1)]. Assessing dietary intake in older populations can also be challenging as individuals may not be involved in their own food preparation, may not be physically able to record intakes, or may have cognitive impairments impacting on their ability to accurately recall intake. It is therefore essential that commonly-used dietary assessment methods are validated in older populations. Based on the uncertainty over the utility of a food frequency questionnaire (FFQ) to determine dietary intake in older people, the objective of this study was to assess the relative validity of assessing fruit and vegetable (FV) intake from a FFQ compared with a food diary (FD).
Methods	A sub-sample of 50 participants (aged >50 years) from the Northern Ireland Cohort for the Longitudinal Study of Aging (NICOLA) completed a FFQ and 4-day FD at two time-points (Month 0 and Month 6). Estimates of FV intake were compared between methods using Spearman's correlation coefficients, examining the percentage of participants classified into the same or adjacent quartile of FV intake, weighted kappa and Bland-Altman plots.
Results	At both time-points, median fruit, vegetable and total FV intake were significantly higher in the FFQ than the FD. Significant positive correlations (all $p<0.05$) were observed between the FFQ and FD estimates of FV intake at both time-points (Month 0, r=0.44, 0.52 and 0.46 for fruit, vegetables, total FV, respectively; Month 6 r=0.49, 0.44 and 0.44, respectively). After individuals were put in fourths (based upon quartiles of total FV portions by FD or FFQ), 79% and 85% of participants were classified into the same or adjacent quartile at Month 0 and Month 6, respectively, while weighted kappa showed fair-moderate agreement between methods for FV intake (weighted kappa=0.33 and 0.44 at Month 0 and Month 6, respectively). Bland-Altman plots revealed a widening in limits of agreements, between the FFQ and FD, with higher FV intakes. Significant positive correlations were observed

	between total FV intakes reported at Month 0 and those reported at Month 6 (r=0.53, p<0.001), with correlation coefficients being stronger for fruit intake (r=0.65, p<0.001) compared to vegetable intake (r=0.36, p=0.01).
Conclusion	While over-reporting is evident with the FFQ compared to the FD, the results show good comparability between the methods in being able to rank older adults according to their FV intake. Analysis of FV biomarkers within this sample will provide a more objective assessment of FV intake by each method.
Abstract published	Yes
Abstract reference	Proceedings of the Nutrition Society 2017; 76 (OCE3), E113

Date	July 2017
Event/location	IAGG, San Francisco, US
Title	Validity of fruit and vegetable intake assessed by a food frequency questionnaire in older adults
Authors	CE Neville, MC McKinley, F Kee, IS Young, CR Cardwell, JV Woodside
Purpose	Accurately assessing dietary intake in older populations is vital to determine the potential role of diet in healthy ageing. Based on the uncertainty over the utility of a food frequency questionnaire (FFQ) to determine dietary intake in older people, the objective of this study was to validate fruit and vegetable (FV) intake from a FFQ, using a food diary (FD).
Methods	A sub-sample of 50 participants (aged >50y) from the Northern Ireland Cohort for the Longitudinal Study of Aging completed a FFQ and 4-day FD (reference method) at two time-points (Month 0 and Month 6). Estimates of FV intake were compared between methods using Spearman's correlation coefficients, cross-classification, weighted kappa and Bland-Altman plots.
Results	At both time-points, median fruit, vegetable and total FV intake were higher (all p<0.001) in the FFQ than the FD. Positive correlations (all p<0.05) were observed between the FFQ and FD estimates at both time-points (Mo 0, r=0.44, 0.52 and 0.46 for fruit, vegetables, total FV, respectively; Mo 6 r=0.49, 0.44 and 0.44, respectively) while weighted kappa showed fair-moderate agreement between methods for FV intake. Cross-classification indicated that 79% of participants were classified into the same or adjacent quartile. Bland-Altman plots revealed a widening in limits of agreements, between the FFQ and FD, with higher FV intakes.
Conclusion	While over-reporting is evident with the FFQ compared to the FD, the results show good comparability in ranking older adults according to their FV intake. Analysis of FV biomarkers within this sample will provide a more objective assessment of FV intake.
Abstract published	No

Date	May 7-11, 2017
Event/location	ARVO Annual Meeting, Baltimore, MD
Title	Factors influencing circumpapillary retinal nerve fibre layer thickness (cRNFLT) in Northern Ireland Cohort Longitudinal Study of Ageing (NICOLA) study
Authors	Paul McCann, Ruth E Hogg, Augusto Azuara-Blanco, Ian S Young, Frank Kee
Purpose	cRNFLT is influenced by ocular diseases such as glaucoma. Demographic and ocular factors including myopia may also influence cRNFLT. The influence of ocular biomechanical properties on cRNFLT has not been reported previously in an adult population based study. We report the association between demographic and ocular biomechanical factors and cRNFLT.
Methods	Cross sectional study: The NICOLA study is an ongoing population-based epidemiological study. The ophthalmic assessment includes: SD-OCT cRNFLT scans, colour stereo pairs of the optic disc and Goldmann-correlated intraocular pressure (IOPg), corneal-compensated intraocular pressure (IOPcc), corneal resistance factor (CRF) and corneal hysteresis (CH) measured using Ocular Response Analyser. Vertical cup-disc ratios (VCDRs) for the first 3001 participants were measured by trained graders who were masked to the cRNFLT measurements. Generalised Estimation Equation (GEE) models were used to enable data from both eyes to be included and multivariable confounder adjusted analysis was performed with the influence of age, sex, IOPg, CH and VCDR explored.
Results	Eyes (n=4375) and participants (n=2486) were included in GEE which demonstrated age (β, -0.139, p<0.001), female gender (β, 2.007, p<0.001), IOPg (β, -0.348, p<0.001) VCDR (per 0.1 increase) (β, -1.0817, p<0.001) and CH (β, 0.366, p=0.022) were significantly associated with cRNFLT. CRF was not associated with cRNFLT in univariate analysis (**r**=0.003, p=0.819) but CH was positively correlated with global cRNFLT in the multivariate analysis.
Conclusion	In addition to age, gender, IOP and VCDR we have identified that lower corneal hysteresis is associated with lower cRNFLT when adjusted for other factors.
Abstract published	Yes
Abstract Ref	Investigative Ophthalmology & Visual Science June 2017, Vol.58, 3136

Date	2017
Event/location	European Congress on Personalised Medicine
Title	Genetic biomarkers in the over 40s in Northern Ireland: evidence from the Northern Ireland COhort of Longitudinal study of Ageing (NICOLA)

Authors	Canadas Garre M, Smyth L, Kee F, Young I, McKnight AJ on behalf of the Northern Ireland COhort of Longitudinal Ageing collaborative group
Purpose	The aim of this study is to identify biomarkers associated with CKD, lipid levels and anthropomorphic traits in older adults in Northern Ireland and to describe a genomic profile of this population.
Methods	This is a cross-sectional study using biomolecular and clinical data from 2,807 patients from the first Wave of data collection in NICOLA. Demographic and clinical information was collected with follow-up interviews planned every two years and health assessments every four years. A range of phenotypes was investigated: CKD (estimated glomerular filtration rate (eGFR), creatinine, cystatin C, CKD stage), lipid levels (total cholesterol, low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and triglyceride (TG) levels), anthropomorphic traits (height, body mass index, waist-hip ratio). DNA was extracted from buffy coats. Genotype data (n=551,839 markers) was generated using Illumina's Infinium CoreExome-24 BeadChips for high-throughput screening on an iScan. Quality control (QC) was performed in PLINK and association analysis (logistic or linear regression) in PLINK and R/SPSS.
Results	Phenotype measures were obtained for 2,529 (kidney measures), 2,543 (lipid levels), and 2,488 (anthropomorphic traits) individuals after QC (Table 1). Age and sex were employed as covariates. Several markers in chromosomes 11, 16, and 19 were significantly associated with lipid levels in this population (Table 2). No association with genome-wide significance was identified for kidney disease or anthropomorphic traits.
Conclusion	Some of the markers identified in this study in older individuals confirm previous associations with lipid levels in other populations. Despite multiple loci being identified in association with eGFR and CKD in both European and non-European populations, those were not replicated in this study, likely as a consequence of the relatively small number of individuals investigated in NICOLA with this phenotype.

Date	2017
Event/location	NIA Biomarker Network Meeting
Title	Analysis of Biomarkers in the Northern Ireland Cohort of Longitudinal study of Ageing (NICOLA)
Authors	McKnight AJ on behalf of the NICOLA collaborative group

Purpose	The NICOLA study was formally launched as Northern Ireland's largest ever public health research project in 2014. This collaborative initiative has finished fieldwork for WAVE 1, collating detailed information on 8,500 local residents over the age of 50 years, with follow-up interviews planned every two years and health assessments every four years. There is a particular focus on multi-omic biomarkers in this relatively early-stage, yet rich resource where additional health and social care information is linked by a unique identifier for each citizen.
Methods	NICOLA created a powerful local biorepository including thirty-four biochemistry-based biomarkers linked to genetic-epigenetic-transcriptomic data. DNA was extracted from buffy coats, while RNA was extracted from PAXgene tubes for all individuals. Genotype data (n=551,839 markers) was generated using Illumina's Infinium CoreExome-24 BeadChips for high-throughput screening on an iScan. Initial quality control was performed in GenomeStudio (v2) with association analysis conducted for a range of phenotypes using PLINK and R. SNPs are being imputed to the Haplotype Reference Consortium. Illumina's Infinium MethylationEPIC BeadChips were employed to quantitatively interrogate >850,000 methylation sites across the genome for 2,000 samples. Data was analysed using GenomeStudio (v2011) with the distribution of methylation levels visualised; concordance rates was excellent (r>0.99), and standard quality control was employed prior to further data analysis. mEPIC data is comparable with Sequenom's EpiTYPER results with a low false positive rate where $\Delta\beta \geq 0.2$. RNA-seq data was generated using Ion Torrent's S5XL™ system with both the whole transcriptome and AmpliSeq™ transcriptome (~21,000 targets) approaches; the AmpliSeq protocol has minimal hands on time for 10 ng of input RNA. Quantitative RNA-seq data was analysed using Torrent Suite and Partek Genomics Suite software, with expression compared to that from microarray and qPCR platforms. This robust approach for creating a biomarker repository and developing pipelines for analysis is feasible for larger multi-centre studies.

2018

Date	Abstract submitted May 2018; acceptance TBC
Event/location	American Society of Nephrology
Title	Genetic and epigenetic analysis in genes affecting mitochondrial function are associated with chronic kidney disease in an older population
Authors	Ruaidhri Cappa[1], Laura J Smyth[1], Marisa Canadas Garre[1], Cassio P de Campos[2], Ryan Skelly[1], Bernadette McGuiness[1], Sharon Cruise[1], F Kee[1], Catherine Godson[3], Alexander P Maxwell[1], Amy Jayne McKnight[1] on behalf of the Northern Ireland COhort of Longitudinal Ageing collaborative group. 1. Epidemiology and Public Health Research Group, Centre for Public Health, Queen's University Belfast, Belfast, United Kingdom

	2. Utrecht University 3. University College Dublin
Abstract	The Northern Ireland COhort for the Longitudinal study of Ageing (NICOLA) is a ten-year project exploring health and lifestyle information linked to an extensive bioresource in 8,500 people over the age of 50 years. Chronic kidney disease (CKD) affects ~10% of the World's population and is more prevalent in older individuals. Optimal renal function is heavily dependent upon efficient mitochondria, therefore genetic and epigenetic features that lead to mitochondrial dysfunction may influence CKD. The discovery cohort comprised 2,567 individuals with body mass index ranging from 18.5- 40 kg/m^2. Genotyping was performed using Illumina's Infinium CoreExome array (n=551,839 SNPs directly typed), with data imputed to the Haplotype Reference Consortium. Methylation data was generated using Illumina's Infinium MethylationEPIC array (866,554 features with single site resolution). PLINK and Partek Genomics Suite were employed to investigate association with eGFR, albumin, urea, and creatinine. Replication was conducted in a 402 independent individuals. SNPs that demonstrated the most evidence for association include an exonic SNP in the mitochondrial genome *MT-TL2* gene (-rs2853498; A12308G; a key SNP defining mitochondrial Haplogroup U) with increased creatinine levels (P= 0.000153, OR= 1.185). SNPs in nuclear genes that influence mitochondrial function include rs77790196 within *SLC39A1* (P= 4.4E-07, OR= 0.0055) and rs12564199 within *JTB* (P= 6.6E-07, OR= 0.006) associated with decreased eGFR. Analysis of epigenetic data identified eight genes demonstrating differential methylation with $p<10^{-8}$ and Δβ 0.2, including *ZBED3*), *ZNF672*, and *AHCTF1* for participants with early stage CKD compared to individuals with CKD stages 3-5. These analyses have identified novel associations linking CKD with SNPs and CpG sites. This may serve as a future basis for the development of predictive multi-omic biomarkers and/or increased understanding of CKD pathogenesis.

Date	23-26th October 2018
Event/location	47th European Symposium on Clinical Pharmacy, European Society of Clinical Pharmacy, Belfast
Title	Assessment of patient adherence to long term medications within a large pharmacoepidemiological study using the dried blood spot technique
Authors	Feras J Jirjees, Gaoyun Chen, James C McElnay School of Pharmacy, Queen's University Belfast, UK

Purpose	Pharmacoepidemiological studies support the rational use of drugs. One of the main obstacles in drawing conclusions relating to safety and effectiveness of medication from this type of study is medication non-adherence, which is a major issue in the management of chronic illness. It is well known that up to 50% of medicines prescribed/dispensed in real life are not taken by patients as recommended by prescribers, with a large proportion of these not taken at all by patients with chronic diseases. The aim of the research is to develop and use a novel direct method to assess medication exposure/adherence in a large cohort study being conducted in Northern Ireland.
Methods	Dried blood spot (DBS) samples collected on Guthrie cards from participants as they join a study. Patients (n=815) who were treated with one of the following drugs was selected: metformin, allopurinol, fluoxetine, bisoprolol, amlodipine and methotrexate. The inclusion criteria of participants are patients with chronic diseases who are ≥ 50 years old. DBS based analytical methods have been developed for the six drugs and/or their metabolites. Fixed volume (15 microliter) DBS samples were used throughout for assay development and for patient samples. Simple solvent extraction approaches were used for four medicines, and solid phase extraction methods used for two drugs. In all cases reversed phase HPLC was utilised with either UV (metformin and allopurinol), fluorescence (fluoxetine and bisoprolol), and mass-spectrometry (amlodipine and methotrexate) detection.
Results	Linear calibration curves were obtained over wide concentration ranges for each of the six drugs, including at levels many times lower than expected steady state trough levels and higher than expected steady state peak levels of the drugs of interest after multiple dosing. All assay methods were shown to have good selectivity, specificity, accuracy and precision according to the international guidelines. A significant proportion of participants (15.3%) within the cohort had no medication of interest in their blood samples, clearly indicating that they were not taking any of the medication at the time of sampling. This was a particular issue for patients using fluoxetine and bisoprolol. Only 57.1% of the patients overall had blood levels within the therapeutic range, indicating that there is significant scope for medication optimisation within the cohort overall.
Conclusion	The results of the present study illustrate, for the first time, the possibility of using a DBS sampling approach to assess adherence/exposure to medicines within a large cohort study. The DBS approach was found to be a straightforward, objective approach to assess exposure/adherence to six drugs.

Date	29th November 2018
Event/location	22nd European Society for Patient Adherence, Compliance, and Persistence (ESPACOMP) conference (http://espacomp.eu/#toc_2), Dublin
Title	Evaluation of adherence to antihypertension medications using dried blood spot approach

Authors	Feras Jirjees, Gaoyun Chen and James C McElnay
Purpose	Adherence to antihypertensive medication is crucial in the control of blood pressure. Indirect assessment approaches have indicated that adherence to antihypertensive medications ranges from 24.3 to 87.6%. The aim of this study was to develop a direct method to assess adherence to the highly prescribed antihypertensive medications amlodipine and bisoprolol, using dried blood spot (DBS) samples and to use the method in a cohort of patients participating in a large cohort study (NICOLA study).
Methods	HPLC assay methods for DBS samples, using mass spectrometry (amlodipine) or fluorescence (bisoprolol) detection, were developed and validated according to ICH guidelines. DBS samples were collected for 503 hypertensive patients who were prescribed amlodipine or bisoprolol in the primary care setting. All participants were ≥ 50 years old. Fixed volume (15 microliter) DBS samples were used throughout for assay development and for patient samples.
Results	The DBS technique was shown to be sensitive and specific for the measurement of amlodipine and bisoprolol concentrations. The limits of quantification of amlodipine and bisoprolol were 0.5 and 4.7 ng/ml, respectively, which are lower than the expected trough level of drug concentrations in blood during routine treatment. The results indicated that 33% of participants had blood concentrations outside the expected therapeutic values, indicating non-adherence. Out of the 503 patient samples, 72 had no drug present. Most of the participants (93.6%) who were prescribed one of the selected medications were receiving three or more medications for the treatment of chronic illness (mean 6.7 medications). Generally, there was no association between exposure and non-exposure to the selected medications and number of medications used by the participants.
Conclusion	The methods developed for measuring amlodipine and bisoprolol concentrations in DBS samples were reproducible, accurate and cost-effective for evaluation of drug exposure in patients and thus in evaluating adherence. Non-adherence in this study was estimated to be high, however, it is within the value that is reported previously using indirect methods of adherence assessment.

Date	October 2018
Event/location	Integrating Genomics and the Social Sciences, Boulder, Colorado
Title	An investigation into the DNA methylation patterns of risk and time preference in older individuals
Authors	LJ Smyth, SM Cruise, I Young, B McGuinness, J Tang, F Kee, AJ McKnight on behalf of the Northern Ireland Cohort for the Longitudinal Study of Ageing collaborative group
Purpose	Risk-preference namely our attitude to risk and to decision making under uncertainty, and time preference, the choice between receiving a smaller and immediate reward opposed to a larger and future reward, are complex traits that have both environmental and genetic determinants. We aimed to examine how an individual's risk and time preferences associate with their epigenetic profiles, specifically DNA methylation patterns.

Methods

8,452 participants were recruited as part of the Northern Ireland COhort for the Longitudinal study of Ageing (NICOLA). Risk preferences were ascertained by asking participants to make a series of choices between two hypothetical income scenarios. Data was collected for 4,564 individuals. Income A, which will with certainty give you £1,500 per month for the rest of your life. Income B, which will give you a 50-50 chance of £3,000 and a 50-50 chance of £1,000/£1,200/£1,300 per month for the rest of your life. In total, 1,656 individuals for whom we had DNA methylation and risk preference data, were included in the analysis; 52% were females and 48% were males; four groups were created (quartiles on the risk preference scale) ranging from "risk averse" individuals to "risk seeking" individuals.

Time preferences were established by asking participants to make choices between a series of hypothetical scenarios. Data was collected for 4,585 individuals. Would you rather have £1,500 now or £1,506/£1,512/£1,518/£1,524/£1,536/£1,548/£1,596 a month from now? Questionnaire and DNA methylation data was gathered for 1,648 individuals; 52% were females and 48% were males. Eight groups were created, ranging from "patient" to "impatient" individuals.

Blood-derived DNA was processed consistently within our single genetics centre. Using the Infinium HD Methylation Assay, MethylationEPIC BeadChips from Illumina evaluated the status of >850,000 CpG sites, promoters and CpG islands. Partek Genomics Suite 7.0 was utilised for data analysis, with standard quality control applied

Results

We compared the distribution of single site DNA methylation levels in the top and bottom quartiles (risk averse vs risk seeking individuals). A total of 393 CpG sites were identified as having significantly different levels of methylation where $p \leq x10^{-05}$, 101 of which were identified in both males and females. Several genes including CALN1, HLA-DPB1, LIMD1, NWD1 and SEPT4 reported multiple significant CpG sites, none of which had previously been linked to risk aversion. Nicotine addiction was established as the pathway with the greatest enrichment score (17.7), where $p=1.9 x10^{-08}$ in males. A subsequent analysis identified that the methylation values of 61 CpG sites displayed evidence of a linear trend across the risk preference scale.

We assessed the methylation levels between the "patient" and "impatient" population groups and identified 94 CpG sites significantly associated with the trait, where $p \leq x10^{-05}$. In total, 16 CpG sites identified were reported in both males and females $P \leq x10^{-03}$. These sites are present within genes including COL1A1, PLEKHG5, STK10 and UXS1, none of which have previously been reported in association to time preference.

Population	Participants	Males (%)	Females (%)
NICOLA total	8,452	3,775 (44.7)	4,677 (55.3)
Risk preference	4,564	2,015 (44.1)	2,549 (55.9)
Risk preference and methylation	1,656	802 (48.4)	854 (51.6)
Time preference	4,585	2,037 (44.4)	2,548 (55.6)
Time preference	1,648	799 (48.5)	849 (51.5)

	and methylation
Conclusion	Epigenetic modifications, including DNA methylation, have not to date been linked to risk aversion and impatience, but may represent important biomarkers of accumulated, but complex genetic and environmental determinants of these traits. Several striking results from this study support further analysis of DNA methylation as an important link between measurable biomarkers and health behaviours. Data from longitudinal cohorts provide the opportunity to monitor the relationship between the two, over time.

Date	October 2018
Event/location	American Society of Nephrology / San Diego, California
Title	An investigation into the DNA methylation patterns of chronic kidney disease in older individuals
Authors	LJ Smyth, SM Cruise, J Kilner, AP Maxwell, I Young, B McGuinness, F Kee, AJ McKnight on behalf of the Northern Ireland Cohort for the Longitudinal Study of Ageing collaborative group
Purpose	Changes in DNA methylation are associated with chronic diseases. The study assessed whether methylation status of CpG sites differs between individuals with and without CKD between the ages of 60 and 79.
Methods	Participants were recruited as part of the Northern Ireland COhort for the Longitudinal study of Ageing (NICOLA), a large-scale population-based prospective cohort study. Estimated GFR was calculated for each individual (n=1,097) using the CKD-EPI formula. CKD stages, based on eGFR, were determined and all individuals with stage 2 CKD (eGFR >60 - <90mL/min/1.73m^2) were removed to increase discrimination between CKD case and control groups. Using the Infinium HD Methylation Assay, MethylationEPIC BeadChips from Illumina, the methylation status of >850,000 CpG sites, gene bodies, promoters and CpG islands were determined in each individual. Blood-derived DNA was processed consistently within our single genetics centre. Partek Genomics Suite 7.0 was utilised for data analysis, with standard quality control applied. In total, 155 individuals had CKD stages 3, 4 or 5 and were compared with 240 individuals with eGFR >90ml/min/1.73m^2 and no evidence of renal disease.
Results	In total, 306 CpG sites were identified as having significantly different levels of methylation in individuals with CKD compared with controls ($p<1x10^{-07}$). Among the genes identified with altered methylation status, several, including *CLU*, *NOS3*, *IQSEC1* and *NPHP4* have been linked with CKD. High concordance between duplicate samples was also observed for this array. Three of the significantly associated CpG sites demonstrated a graduated increase in the methylation fold change with worsening renal function i.e. comparing control individuals with persons having CKD stages 3, 4 and 5 respectively.
Conclusion	Epigenetic modifications, such as DNA methylation, may represent important biomarkers for the loss of kidney function in individuals with CKD. Data from this longitudinal cohort study provides the opportunity to monitor and assess the relationship between methylation status and CKD over time with a view to identifying new biomarkers or expanding knowledge of those previously identified CKD biomarkers.