

Understanding the disorders of the RAS-MAPK pathway
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Background to the Study

Germline disorders of the RAS-MAPK pathway arise due to dominant mutations which cause RAS-MAPK pathway dysregulation [1]. Cardio-facio-cutaneous (CFC) and Costello syndrome (CS) are the most severe conditions in this group of disorders, and have overlapping features, including congenital heart anomalies, growth failure and learning disability, all of which may be severe [2]. Such features also occur commonly in other RAS-MAPK disorders including neurofibromatosis type I and Noonan syndrome, which jointly affect over 1 in 1000 of the population [2]. Progressive cardiomyopathy, epilepsy, scoliosis and other bone problems can occur in CFC and related disorders [2]. The RAS-MAPK pathway has been studied for many years in view of its key role in oncogenesis. Its signalling cascades result in activation of extracellular signal-related kinases, which, translocating to the nucleus, upregulate transcription of many genes influencing cell cycling and apoptosis [3]. Somatic mutations of genes of this pathway that are found in cancer, like those found in the germline disorders, show altered kinase activity [3].

Genetics of germline RAS-MAPK disorders

There are many different genes that are now known to cause RAS-MAPK pathway disorders, but a proportion of patients with a presentation strongly suggestive of this type of condition do not have an identifiable mutation at present: as many as 40% of patients with a clinical diagnosis of CFC or Noonan syndrome have no identifiable mutation currently [2].

For the individuals and families we see in clinic, one main reason for trying to find the genetic basis for their or their child's condition is so that they can find out accurate information about the risks of recurrence in further children, any risks for the extended family and the possibilities of a prenatal test in another pregnancy. A further important reason for identifying the genetic basis of an individual's condition is that it may in future predict their response to potential therapies for these disorders.

Aim of this current study

1. To characterise the clinical presentations of a group of patients with germline disorders of the RAS-MAPK pathway, including patients with Noonan, Costello syndrome, and those with features of cardio-facio-cutaneous syndrome, a poorly-understood but frequently severe condition which is due to dysregulated RAS-MAPK pathway signalling. The aim for the number of patients to be recruited is 100, but this will depend upon how many families wish to take part in the research.
2. To search for the genetic basis of the clinical presentation in patients with CFC and related disorders using newer types of genetic analysis including a) high resolution microarray analysis and b) high throughput sequencing. This will be of benefit to the individuals and families themselves, and will also be useful in guiding the development of new diagnostic testing strategies for this group of disorders.

3. To make available any findings from this study so that they can be used to aid patient management and genetic counselling in the families involved.

Plan of investigation

This study will investigate the phenotypes of a group of patients with clinical presentations suggestive of a germline RAS-MAPK pathway disorder. Some of these will have a previously confirmed molecular diagnosis, whilst others will not.

a) The identification of potential participants and recruitment of patients to the study will be via clinical genetics colleagues. Geneticists who have referred samples to the Manchester Regional Genetics Laboratory for genetic testing of CFC or CS will be contacted in this regard. The study will also be publicised via the support groups for these conditions, and potential participants will be invited to contact the investigators, giving permission for their geneticist to be contacted for further details to assess their eligibility for inclusion in the study. Interested potential participants will receive (either via their geneticist or by post) a patient information sheet and consent form with a self addressed envelope in which to return this.

Capacity to consent to involvement in the research will be made in accordance with the British Psychological Society guidelines checklist (issued 2008, see reference 4) jointly by the participant's geneticist and the research team. The former will assess on the basis of their knowledge of the patient and his or her level of function, and the latter on the basis of ability to understand the purpose of the study and retain this information long enough to make a considered decision whether or not to participate. For potential participants with limited reading skills, extra help will be offered with verbal explanation of what is involved. For any participant where there remains a question over capacity to consent, advice and opinion will be sought from the individual's next of kin or general practitioner.

Adults who have capacity to give informed consent to participation will be asked to sign a consent form if they wish to enter the study. For potential participants who are children, parents/guardians will be asked to give consent to their inclusion. For vulnerable adults, who will be few in number but an important group to include with respect to the long-term natural history of these conditions, information will be provided in a format that they can understand, and their capacity to give informed consent will be assessed by the doctor making the initial approach. Where a potential participant does not have capacity to consent to involvement in the study, their parent, guardian or advocate will be asked to consider whether or not taking part in the study is in their best interests. If this person and the doctor making the initial approach concur that involvement in the study would not be harmful, and could be potentially beneficial to the individual, then the parent, guardian or advocate will be sent an assent form to be returned in the same way as the parental consent form for children.

Once consent has been granted, we will contact the patient's clinical geneticist for clinical details. Where patients will have had previous DNA studies and DNA has been stored as part of their clinical care, we will seek to use these samples if possible rather than asking participants to undergo further venepuncture. Samples from the parents will also be requested, where this is possible.

Patients will be asked if they consent to their clinical details being entered on a research database and anonymous data and photographs being reviewed by a panel of dysmorphologists. They will be asked to consent to the use of their DNA samples and any tissue samples that may become available for genetic studies of their condition. If consent is granted, the patient's GP will be informed about his or her participation in the study.

b) A secure database will be set up, to record the details of the patients, clinical information and results of molecular testing. This will be a Microsoft Access database, which will be password protected and accessible only by the investigators and the genetics research co-ordinator who would only need access to details in an emergency, if the investigators were absent. Names will be kept on the database as it will be necessary to refer back to individual families if genetic changes are identified. All other identifying data will be kept on paper proformas in a locked filing cabinet.

c) Participants will be offered an appointment either in clinic or at their home, whichever is more convenient for them. The content of this visit will consist of clinical history taking and examination (similar to that which would be undertaken as routine clinical genetic work up). Participants aged 6-16 would also be offered a psychometric assessment using a well-validated set of tests, as listed below:

| Measure | Cognitive Function |
|---|-------------------------------|
| Child Measures : | |
| Dependent on child's developmental level | |
| Above age 6 : | General Intelligence |
| WASI (32) | |
| (Wechsler Abbreviated Scale of intelligence | Vocabulary |
| Vocabulary | Visual processing |
| Block Design | Verbal reasoning |
| Similarities | Visual information processing |
| Matrix reasoning | |
| Below age 6: | General Intelligence |
| Mullen Scales of Early Learning | Visual perceptual ability |
| Visual Reception Scale | Receptive Language & Visual |
| Receptive Language Scale | visual information processing |
| | Expressive Language |
| Expressive Language Scale | |
| Cambridge Neuropsychological Testing Automated Battery | |
| (CANTAB) (Luciana, 2003) | |
| Paired Associate Learning (PAL) | Visual Spatial Learning |

If child's developmental level is above the age of 6:

Test of Everyday Attention for Children (TEA-Ch) (Manly et al. 1999)

Score!

Sky Search

Creature Counting

Sky Search DT

For all children:

Test Observation Form for Ages 2-18 (TOF) (McConaughy & Achenback, 2004)

Sustained Attention
Selective Attention
Attentional control/Switching
Divided Attention

Attentional problems and
language problems

**Judgment of Line Orientation (JLO) (Benton et al. 1976)
Continuous Performance Test (CPT-II or TOVA) (Conners
2000; Greenberg 1999)**

Visual Spatial Skills
Sustained attention and
concentration

**Autism Diagnostic Observation Schedule-2 (ADOS-2) (Lord
et al. 2012)**

Social Interaction,
Communication and
Language
Repetitive Behaviours

**Diagnostic Analysis of Nonverbal Accuracy DANVA(35)
(Norwicky & Duke, 1994)**

Receptive and Expressive
Nonverbal Processing

Parental Measures:

**Parent Version of the Conners ADHD/DSM-IV Scale
(Conners, 1997)**

**Behaviour Rating Inventory of Executive Function (BRIEF)
Parent Form (Gioia, Isquith, Guy, Kenworthy 2000)**

ADHD

Executive Functioning

**Autism Diagnostic Interview- Revised (ADI-R) (Lord et al.
1994)**

Social Interaction,
Communication and
Language
Repetitive Behaviours

Teacher Measures:

**Teacher version of Behavior Rating Inventory of Executive
Function (BRIEF) (Gioia, Isquith, Guy, Kenworthy 2000)**

Executive Function

Test duration

The PAL from Cantab tests will take approximately 15 minutes each to administer, JLO 10 minutes, the TEA-Ch test (if appropriate) will take about 25 minutes altogether, ADOS-2 around 40 minutes and the CPT-II 14 minutes. Depending on the child's developmental age, the WASI or the Mullen will be carried out. The WASI will take around 30 minutes and the Mullen will take around 40 minutes. Overall assessment time for the child will be just over 2 hours. The TOF will also be completed; however this is an experimenter observation form, therefore, no time burden for the child.

The parental questionnaires should take 5+10 minutes (Conners' scale) and BRIEF 15 minutes (provided parents don't have learning problems themselves) and the ADI-R approximately 2.5 hours. Overall assessment time for parents will be around 3 hours. The assessment of the children and parental interview will be carried at the same time, unless the parents request an alternative arrangement.

d) DNA samples will be forwarded to the DNA laboratory at St Mary's Hospital and logged in on the LIMS information system which is password-protected. Each sample will be given a number which will be used from then on for identification purposes in the laboratory rather than using the patient's name.

Where a new blood sample is being taken, and if consent is granted, part of this sample will be used to make a cell line. This will enable further studies to be undertaken regarding the effect of RAS-MAPK pathway signalling in living cells. It will also be a source of further DNA from the individual, should the original sample be exhausted (thereby preventing the need for repeat blood sampling).

Where a participant is undergoing a surgical procedure, samples of tissue other than blood may become available. If this were the case, we would seek consent from the participant or their parent/guardian to obtain samples of any material made available in this way (for example, skin biopsy). No samples would be taken that would not normally have been accessible in the course of the procedure.

e) The clinical features of participants will be systematically assessed, in order to better understand the range of problems encountered by patients with these disorders. Information will be gathered from the details provided by the referring clinician, and by history taking and clinical examination in person (by Dr Burkitt Wright). Clinical photographs will also be used to record facial and other features apparent on examination. These will then be assessed by the investigators and a consensus reached on the features that are present. Dr Kerr will be blinded regarding the genotype of these patients, in order to minimise bias in this part of the data analysis.

f) Microarray studies will be carried out on patient DNA using the Affymetrix SNP6.0 platform to look for small chromosomal imbalances in any patients who have not undergone molecular cytogenetic studies of this type previously. Data will be analysed using appropriate computer software in the array laboratory at St Mary's Hospital. If an imbalance is identified, FISH or QFPCR analysis (for small imbalances) will be used to

verify the abnormality. Parents will also be screened to check whether the abnormality has occurred de novo, as this would be of greater significance. The deleted/duplicated region would be checked against Ensembl and other databases to identify whether any genes lay within or close to the region in question and these would be considered as candidate genes for future studies. Care will also be taken to look at genes just outside but close to any region of imbalance which could be implicated due to a positional effect.

g) A targeted next-generation sequencing approach will be used to screen genes that could be implicated in germline RAS-MAPK pathway disorders. The genes to be screened will be those known or predicted to be involved in RAS-MAPK signalling, and will include those in areas of the genome where chromosomal rearrangements/deletions/duplications have been identified in patients with suggestive phenotypes. A next generation high throughput sequencer has recently become available through the Manchester Biomedical Research Centre. Where possible, samples from affected individuals will be run in parallel with those from their parents, so that possible causal changes identified in the affected person can be verified as being either de novo or inherited. The results of testing of parental samples will only be analysed for genes in which possible changes have been identified in the affected individual, minimising the (already small) risk that results of adverse significance to the asymptomatic parent would be obtained. In the unlikely event of non-paternity, for example, being suggested by the results of genetic testing, this information would not be disclosed to participating individuals. The offer of genetic counselling (through Manchester Regional Genetics Service or the participant's local genetic service) will be available for any participant in this study.

Results

Any positive results will be confirmed in a CPA accredited diagnostic laboratory before being fed back to the families concerned and their clinicians. Findings of importance to the genetic community will be presented at appropriate meetings and written up for dissemination in genetic / paediatric journals and as part of the thesis to be submitted by Dr Burkitt Wright to the University of Manchester for the degree of PhD. No identifying patient data will be used in presentations/publications. Explicit consent for the use of patient photographs will be obtained.

At the end of the study we will write to all families to tell them that the study has finished and inform them of the outcome, irrespective of whether there are positive findings for that family or not.

Funding

Funding for this project has already been obtained: the chief investigator (Dr Emma Burkitt Wright) has been awarded a research training fellowship by the Wellcome Trust (May 2010-April 2013).

References

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3. Yoon S, Seger R (2006). The extracellular signal-regulated kinase: multiple substrates regulate diverse cellular functions. Growth Factors. 24: 21-44.
4. Conducting Research with people not having the capacity to consent to their participation: a practical guide for researchers (page 49) Prepared by Catherine Dobson on behalf of the Mental Capacity Act Working Party (2008). British Psychological Society, London.