Brazil's cancer curse

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The startling discovery that hundreds of thousands of Brazilians have a genetic mutation that undermines their ability to resist cancer is helping labs worldwide in their search for new treatments for the disease. Sue Armstrong reports.

Pedro Gomez is a short, powerfully built man in his 60s with the ruddy face and sun-tanned arms of an outdoor worker. He is wearing a short-sleeved black shirt, jeans and a baseball cap. Gomez is worried about a small lump on his finger, he tells the doctor, cancer geneticist Maria Isabel Achatz. Taking his hand in hers to get a closer look, Achatz talks to him gently then leans forward to inspect another small lesion behind his ear.

Gomez is one of Achatz's regular patients at the A C Camargo Cancer Center in São Paulo, Brazil. He is extraordinarily susceptible to cancer. So too are many members of his extended family; cancer is so common among them – and premature death so painfully familiar – that until they learned very recently of the cause, some believed their family was cursed.

Gomez's is not the only family affected. The 'curse' afflicts hundreds of thousands of people in Brazil. One of the highest-profile was José Alencar, the country's popular and charismatic Vice President under Luíz Ignácio 'Lula' da Silva. Alencar died in 2011 after being diagnosed with cancer in 1997. Over the years, as tumours spread relentlessly throughout his body, he underwent more and more operations in Brazil and the USA, having a kidney, most of his stomach and large chunks of

his bowel removed. The Vice President talked candidly about his disease and used his own experience to advocate for early detection of cancer.

What Gomez, Alencar and the other Brazilians have in common is a single change in their DNA – a mutation in a gene called p53 that undermines their ability to resist cancer.

p53 has turned out to be the most important single gene in cancer, and has been one of the most popular areas of study in the history of molecular biology. The gene was discovered in 1979 by David Lane, working at the Imperial Cancer Research Fund in London, and coincidentally at exactly the same time by three other groups working independently in the USA and France, and led by Arnold Levine, Lloyd Old and Pierre May.

p53 is a tumour suppressor. Its job is to protect us from cancer by making sure that, when our cells divide as part of routine growth and maintenance of our bodies, they do so without making dangerous mistakes. If the DNA – the cell's operating instructions – is damaged or not copied faithfully as it divides to produce new 'daughter' cells, p53 stops the cell in its tracks and sends in the repair team before allowing the dividing cell to proceed. If the DNA damage is irreparable, p53 puts the cell into a state of 'replicative senescence' so it cannot divide again; or else it instructs the cell to commit suicide lest it run amok.

When one reflects that, over an average lifespan, a person will experience some 10,000 trillion cell divisions, and that it takes just one rogue cell to start a tumour, the importance of this gene becomes clear. Because of its vital role in quality control, David Lane nicknamed p53 'the guardian of the genome'. The gene itself is disabled by mutation or some other faulty mechanism in almost every case of human cancer.

Most often this corruption of p53 occurs spontaneously in individual

cells or tissues that have sustained some damage in the rough and tumble of living, and this can set them on the path to cancer – a risk that increases the longer one lives. But some people are born with corrupted p53 in every cell of their body, and are extremely vulnerable to cancer from their earliest days.

Li–Fraumeni syndrome, as the condition is called (it was first reported by Frederick Li and Joseph Fraumeni in 1969), has several notable characteristics. Affected people are particularly prone to sarcomas of the soft tissues and bones, cancers of the brain and breast, leukaemias, and carcinomas of the adrenal glands. They usually develop cancer at an exceptionally early age, and until the early 2000s, when Maria Isabel Achatz began to see patients in her cancer genetics clinic, Li–Fraumeni syndrome was thought to be very, very rare.

As a young woman, Achatz left her family home in Rio to study art in Paris. But a trip to India with fellow students during a college break was a life-changing experience. While visiting a leprosy colony in a remote desert location on the Kashmir border, she met the missionary who ran it: Mother Teresa. "It was an amazing encounter, and I thought, 'Well, I just have to go back and do something [more worthwhile],'" says Achatz. On her return to Brazil she studied medicine, opting eventually to specialise in genetics.

Among the first patients she saw in the clinic were a number of people who had already suffered multiple bouts of cancer, often starting in childhood, and their tumours were typical of the cancers most commonly seen in people with Li–Fraumeni syndrome. What's more, when she drew up detailed family trees with her patients – a routine practice in genetic counselling with certain diseases – she uncovered trails of cancer among their relatives, often reaching back generations. They had all the hallmarks of Li–Fraumeni, but Achatz was perplexed: "It really struck me because this was considered to be a very rare syndrome around the

world. There were only 280 families described in the medical literature at that time, and I had 30. So I thought, 'Either I'm over-diagnosing or something unique is happening here.'"

Her colleagues in Brazil were as intrigued as she by what she was seeing in her clinic and encouraged her to take her story to a cancer conference in France in 2002. There Achatz caught the attention of Pierre Hainaut, a tall, bespectacled Belgian who worked at the World Health Organization's International Agency for Cancer Research in Lyon. Hainaut was custodian of a database of all the different mutations in the p53 gene recorded in the medical literature, and the types of cancer with which each mutation was associated. Aware from his records of the extreme rarity of Li–Fraumeni syndrome, he was fascinated by Achatz's case notes. He persuaded the young doctor to return to France with blood samples from her Brazilian patients and work with him on identifying exactly what was wrong with their p53 genes.

The two researchers were in for some surprises. Very few of the patients had 'classic' mutations in p53 associated with Li–Fraumeni cases elsewhere in the world; Achatz's initial conclusion was that she had over-diagnosed the syndrome. But closer inspection revealed that many of her patients had a p53 mutation that was outside any of the hotspots on the gene known to be most vulnerable to corruption. What's more, all the patients with this unique mutation carried the exact same copy of the gene.

Some 1,200 km to the south of São Paulo, Patricia Prolla – a fellow cancer geneticist working in Porto Alegre – was also seeing an unusual number of patients with Li–Fraumeni syndrome. And when these turned out to have the same p53 mutation as Achatz's patients, Prolla and Hainaut resolved to find out how prevalent it might be in the general population. They tested blood from a large sample of apparently healthy women enrolled in a preventive breast screening programme at the Porto

Alegre clinic and found, remarkably, that nearly one in 300 had the faulty p53 gene. This startling result was confirmed by a screening programme among nearly 200,000 newborn babies in the nearby state of Parana, where doctors had been finding especially high rates of adrenal gland cancer in small children. Again, it was linked to the same p53 mutation.

"That means that the population of south and south-eastern Brazil has an immense number of Li–Fraumeni carriers, probably more than 300,000," says Achatz. "People are just not aware of this, so probably many cancers that are occurring in the population in general are due to this mutation and people just don't realise."

And it's not just Brazil. Very recently, the same mutation in p53 has also been found in neighbouring Paraguay, where geneticists randomly tested 10,000 samples of blood from newborn babies. The results suggest that here too several thousand people could be living with Li–Fraumeni syndrome.

If thousands of people share an identical genetic mutation, it's not due to coincidence. There must have been a 'founder', one man (so the thinking goes) with Li–Fraumeni syndrome who passed his mutant gene on to his offspring, setting the ball rolling down the generations.

We don't know the name of this original carrier, the common ancestor of all today's carriers, nor where he came from – he may have been an immigrant from Europe. The rogue gene is believed to have travelled along the routes opened from the coast to the interior by early explorers, settlers and military men. One appealing notion is that the founder was a tropeiro, one of a band of travelling traders who journeyed by mule between the scattered settlements, carrying goods and gossip and mail in the 17th and 18th centuries. Away from home most of the time, a tropeiro would likely have had a string of girlfriends along the road,

offering an ideal opportunity for passing on his genes. One of Achatz's biggest Li–Fraumeni families can trace its history back to tropeiro ancestors.

But Hainaut believes a more likely candidate for this 'patient zero' would be either a military man or a bandeirante – one of the ruthless adventurers who raided inland for native slaves to trade and to search for precious minerals. When gold was discovered in the 17th century, the rush was on to claim the territory for Portugal before the Spanish could do so. Both the bandeirantes and government servants set to with feverish intent, carving out routes to the interior and creating new settlements along the way. A distribution map of the founder mutation corresponds closely with these routes.

If the founder had been carrying one of the p53 mutations of classic Li–Fraumeni syndrome, it's unlikely his genes would have spread so far. The lifetime risk of developing cancer for people with such mutations is around 90 per cent, and people born with such pernicious genes have a much-reduced chance of raising a family. (This is one reason why so few cases had been recorded in the medical literature when Achatz first started to see the syndrome in her clinic.) The lifetime risk of cancer for those with the Brazilian mutation is 50 to 70 per cent and, paradoxically, it is this milder character that has enabled it to spread so far and affect such huge numbers of people. Most carriers survive long enough to pass the gene on to their children, and some never develop cancer at all.

The A C Camargo Cancer Center is in a run-down neighbourhood of São Paulo, with narrow streets, small shops and open-fronted diners. In its modern laboratories, which dominate the skyline, is stored the biggest collection of tumour samples in the region – 30,000 scraps of tissue preserved in paraffin wax blocks, meticulously labelled and filed in cabinets.

By studying these tumour samples, Achatz and her colleagues are trying to understand how p53 works in people rather than lab dishes or mice, and how cancer develops when the gene stops functioning properly. For example, one of Achatz's patients is a woman who, by the age of 18, had developed 14 different tumours. Samples have been taken from many of these tumours, and now the researchers can examine the differences between the DNA of the cancerous tissue and that of the woman's normal cells.

Meanwhile, Achatz's colleague at A C Camargo, Fernanda Fortes, wants to know why children with the Brazilian p53 mutation have at least a ten-fold higher risk of adrenal gland cancer than the general population. And, as not all children with the mutation develop this cancer, what tips the balance in those that do? By analysing as many tissue samples as possible from these children, Fortes is hoping to find out. She already knows that the acidity in their tumour cells is higher than normal. And she knows that this is significant – but how significant and in what way? Is this higher acidity in general a cause or a consequence of malignancy?

This is part of a much bigger topic that is exciting the p53 research community right now: the role of the metabolism in cancer, for it turns out the tumour suppressor is a major player in this arena too.

That the metabolism in cancer cells is highly abnormal is not a new discovery. In the 1920s, the German biologist and medic Otto Warburg noticed that cancer cells consume glucose at an enormous rate. He found that whereas most normal cells break down glucose and shunt its products into the mitochondria – the powerhouses of the cell – where they are burned in the furnace to produce energy, tumour cells partially suppress the activity of the mitochondria and use much of the glucose to create the building blocks of new cells. This metabolic process, known as aerobic glycolysis, takes nearly 20 times as much glucose as mitochondrial respiration to produce the energy that cells need – hence

cancer cells' voracious appetite for glucose.

Warburg believed this altered metabolism was the cause of cancer, and said so in a 1956 paper. But his provocative theory was soon overshadowed by the molecular biology revolution, as excited scientists began to look for the causes of everything in our DNA. The excessive appetite for glucose (the so-called Warburg effect), they said, was a consequence of malignant transformation of cells, not a driving force. But now evidence is mounting that metabolism does play an active part in tumour development after all. Recent work on p53 in particular, says Hainaut, points to the fact that metabolic factors are "absolutely fundamental to the biology of cancer".

There had been clues around since the 1990s that p53 is involved in metabolism, but it wasn't at all clear how this fitted the picture of the gene as a tumour suppressor. In 2005, however, scientists at the US National Institutes of Health compared the endurance of normal mice with ones whose p53 gene had been deleted. The mice were put into a bucket of water, and those lacking p53 went under much more quickly than the normal ones: clearly they were having difficulty generating enough energy to keep afloat. So, what was going on?

At her lab at Glasgow's Beatson Institute, Karen Vousden and her fellow researchers have discovered that, in the normal course of events, p53 plays a subtle role behind the scenes. It's not just watching and waiting to stop or kill potentially dangerous cells, but is actually helping cells to avoid or survive things that might damage them – that is, things that might trigger its anti-tumour response. In other words, p53 is playing a double game: it promotes survival under some conditions, but when it senses things are getting out of control, it calls in the death squad.

The way that p53 promotes survival, explains Vousden, is as a regulator of metabolism, by helping cells cope with fluctuations in the fuel supply.

"This might be something that happens all the time, and you wouldn't necessarily want to kill every cell that just transiently doesn't have enough glucose. So in those situations, it's pretty clear p53 helps cells survive. And it does so by allowing the cell to reorganise its metabolism."

As a basic regulator of metabolism, p53 helps cells resist the glucose-guzzling, inefficient Warburg effect except in emergencies. It also helps clear away free radicals – the corrosive by-products of burning sugar for energy in the mitochondria – thus encouraging the survival of cells by limiting the damage these particles can do to DNA. But if the tumour suppressor isn't working, harmful free radicals can proliferate, and corrupted cells are free to hijack the metabolic machinery and switch over to glycolysis, which enormously boosts their ability to replicate. This is cancer in the making.

This line of research into the metabolic abnormalities of cancer offers some tantalising prospects for patients. For example, what if we could raid the medicine cabinet for drugs that already exist for metabolic diseases and repurpose them as new treatments for cancer? "You wouldn't even need to do clinical trials for safety," points out Vousden, "because these drugs have already been used in millions of humans for years."

It's an idea many labs around the world, including her own and Hainaut's in France, are already exploring with metformin, the most widely prescribed drug for diabetes, which targets faulty glucose metabolism. People with diabetes are usually at increased risk of cancer, but doctors started noticing that the cancer risk in long-term users of metformin seemed to be lower even than that of the non-diabetic population. Could the drug be having a protective effect? Experiments in the lab showed that it is indeed toxic to cancer cells.

"There are good and bad points," cautions Hainaut. "Metformin will be easy to introduce into cancer treatment because it's already on the market and there's a lot of experience of giving it to patients: it's safe, proven, easy to administer. It has all the characteristics to make a quick hit in cancer treatment if it has a positive effect. But in terms of addressing the glucose weakness of cancer cells, it's not that strong."

Metformin is already being tested beyond the lab, with clinical trials of cancer patients in many centres around the world, and Hainaut is encouraging Achatz to try it with some of her patients too. But doctors and scientists alike are acutely aware of the sensitivity of their research among Brazil's Li–Fraumeni families, and the danger of exciting premature hopes in people desperate for breakthroughs.

Since the identification of the mutant p53 gene in so many members of Pedro Gomez's large family, they have been struggling – each in their own way – to cope with the implications for themselves and their loved ones. Gomez's brother, mayor of a small town on the outskirts of São Paolo, took the blood test but balked at learning the results. It was only when his daughter was diagnosed with breast cancer on the eve of her wedding day that he realised he could not hide from the truth. The wedding was postponed while she recovered from a double mastectomy, and today she bullies her father into going along with her for their annual screenings at A C Camargo.

Two of the mayor's nieces are also carriers of the mutant gene. One says of her diagnosis, "It changed my life for ever; it has made me really crazy." She dreads her annual check-ups, which are time-consuming, invasive and stressful as she waits for the results, always expecting bad news having lost her mother to breast cancer. She frets for her young son, whom she has not yet had tested, and frets too over the morality of having more children, which she and her new husband badly want, and about the possibility of losing her ovaries, womb or breasts to cancer

before she can do so. Her cousin, who also wants children, is more philosophical: what will be will be, she shrugs. When she received the news that she had the mutation, the shock she might have felt for herself and her father, who received his test results at the same time, was overwhelmed by concern about her mother's intense distress for her family.

Achatz is acutely aware of the emotional struggles of the Li–Fraumeni families she sees every day in her clinic. "It's very clear to me that I'm in science to treat my patients," she says. "Everything I do goes back to how it affects them."

So, what of the prospects for the diabetes drug? "Between the proof of principle that metformin works in humans and knowing how to deliver it in the right conditions there are still a lot of steps," cautions Pierre Hainaut. "But I am seriously hopeful it will work – at least for the Brazilians."